Comments on the
DRAFT TOXICOLOGICAL REVIEW OF METHANOL
(CAS No. 67-56-1)
In Support of Summary Information on the
Integrated Risk Information System (IRIS)

In Response to Federal Register Notice of January 12, 2010 (75 FR 1617)

Prepared for:

National Petrochemical and Refiners Association
Washington, DC

Prepared by:

Joseph V. Rodricks, Ph.D., DABT
Duncan Turnbull, D.Phil., DABT
ENVIRON International Corporation
Arlington, Virginia

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INTRODUCTION

This document presents comments prepared on behalf of the National Petrochemical and Refiners Association (NPRA) with regard to the EPA draft Toxicological Review of Methanol published in the Federal Register on January 12, 2010. In our view, EPA has given only superficial consideration to the very real issues that we and others (including neutral academicians and officials from public health agencies of the U.S. and European Union) previously raised pertaining to studies conducted by the European Ramazzini Foundation (ERF). Several of those uncertainties pertain to criticisms which, if found to be true, would undermine the validity of several ERF studies, including an ERF study of methanol (Soffritti et al. 2002) that EPA considers critically important to the conclusions of the draft Methanol Assessment. Much of the information and data that EPA purports to rely on in dismissing the criticisms of the ERF studies is, in many cases, incorrect or misleading. Sound scientific and regulatory policy requires that, for all ERF studies relevant to the Methanol Assessment, EPA should:

(i) obtain full study documentation of the type available for National Toxicology Program (NTP) studies;

(ii) evaluate the studies using NTP criteria;

(iii) give little weight to any study for which adequate documentation of conduct and results is absent or for which documentation is inconsistent; and

(iv) thoroughly evaluate the role of infection in tumor production in the Sprague-Dawley (SD) rats used by the ERF, preferably including histopathology peer review by pathologists experienced in diagnosing mycoplasmosis and, if possible, to perform PCR analysis of retained lung tissue samples to identify mycoplasma.

The remainder of these comments sets forth analyses demonstrating the need for these actions.

CRITICAL ISSUES REGARDING ERF STUDY

As a response to the uncertainties that have been raised concerning ERF studies, including significant evidence that infection has likely resulted in the misdiagnosis of some lesions observed in the rats used by the ERF, the Methanol Assessment gives considerable attention to two issues: 1) the results of a limited review of self-selected slides from an ERF aspartame bioassay performed by National Institute of Environmental Health Sciences (NIEHS) pathologists (Hailey 2004); and 2) what the Agency describes as evidence that the incidence of hemolymphoreticular tumors is rare in ERF Sprague-Dawley rats. We begin these comments by showing that the Agency's analysis of these two issues is superficial and misleading. We also present comments explaining other uncertainties and criticisms that EPA does not resolve and that have the potential to undermine the validity of several ERF studies. Finally, we urge EPA to review the publications attached to this document, which provide in-depth analysis of many concerns associated with bioassays conducted at the ERF.

The NIEHS Review of Slides from the ERF Aspartame Bioassay

The Draft Toxicological Review contains several discussions of the results of a limited review of a small set of ERF-selected slides from an ERF aspartame bioassay that NIEHS pathologists performed in 2004 (Hailey 2004; included with these comments for the convenience of the reader as an Attachment). The discussions in the Draft Toxicological Review misrepresent the findings and significance of the limited review conducted by NIEHS. For example, the Draft Toxicological Review states:

> "An NIEHS PWG (Hailey, 2004) has confirmed the ERF diagnosis of the several lymphomas, including three lymphomas from the lung, thymus and medullary lymph node and mesenteric lymph node that were characterized by ERF as "lympho-immunoblastic." (USEPA 2009, at 4-18)

This statement is inaccurate and misleading. First of all, the NIEHS review of a few slides selected by ERF staff cannot be considered to represent a valid Pathology Working Group (PWG) since the NIEHS reviewers did not have the opportunity to select and examine representative samples of all tissues. Indeed, Hailey (2004) himself noted that "this review was not considered a 'peer review' of the pathology data from the study." More importantly, EPA's conclusion that the NIEHS review "confirmed" the ERF findings is erroneous. Table 1 shows a comparison of the ERF diagnosis of the few selected slides that were reviewed compared to the NIEHS PWG majority diagnoses.

Considering all of the slides reviewed, the majority of NIEHS diagnoses agreed with those of the ERF in only about one-third of the tissue lesions examined. The NIEHS worksheets identify 78 slides. In a few cases, there were multiple slides of the same tissue/lesion, and in some cases, more than one tissue/lesion was on a single slide (e.g., left and right kidneys). Among these slides are 61 lesions identified by the ERF as neoplasms. Of these 61, the NIEHS pathologists agreed on 23 diagnoses, including 5 lymphomas about which they said "the NTP does not routinely subdivide lymphomas into specific histological types as is/was done by the ERF, however the PWG accepted their more specific diagnosis if the lesion was considered to be consistent with a neoplasm of lymphocytic, histiocytic, monocytic and/or myeloid origin." In another 21 cases (including 3 lymphoid neoplasms), the NIEHS agreed that there was a neoplasm present, but bit did not confirm the specific type identified by the ERF. In the remaining 16 cases, the PWG consensus was that there was no neoplasm of any kind.

Of 21 non-tumor lesions identified by the ERF among the slides reviewed, the PWG agreed on 7, partially agreed on 7 (for example, simple hyperplasia rather than "papillary hyperplasia with marked atypia"), and disagreed completely on 7 (for example, nothing versus "gliosis"). See Table 1. In sum, agreement on less than half of the relatively few slides reviewed cannot fairly be interpreted as "confirm[ation of] the ERF diagnos[e]s."

TABLE 1
Comparison of ERF and NTP PWG Diagnoses of Selected Lesions from the ERF Aspartame Study

Animal #	Tissue	RF Diagnosis	PWG Majority Diagnosis	Agreement	
				Tumor	Non-tumor
201	Mammary gland	Adenocarcinoma	Fibroadenoma	X	
201	Mammary gland	Adenocarcinoma	Fibroadenoma	X	
202	Mammary gland	Adenocarcinoma	Fibroadenoma	X	
118	Cranial nerves	Malignant Schwannoma	Agree	Agree	
118	Orbital cavity	Invasion of malignant Schwannoma	Agree	Agree	
216	Cranial nerves	Malignant Schwannoma	Agree	Agree	
216	Orbital cavity	Invasion of malignant Schwannoma	Agree	Agree	
83	Brain	Malignant glioma?	Gliosis	XX	
118	Brain	Oligodendroglioma	Glioma	X	
118	Pituitary gland	Adenocarcinoma	Pituitary tumor, malignant	X	
284	Brain	Multiform glioblastoma	Autolysis	XX	
332	Brain	Oligodendroglioma	Mixed glioma	X	
382	Brain	Oligodendroglioma	Gliosis	XX	
441	Brain	Malignant meningioma	Malignant meningioma	Agree	
487	Brain	Glial tumor	Nothing	XX	
560	Brain	Malignant meningioma	Granular cell tumor	X	
565	Brain	Gliosis	Nothing		XX
748	Brain	Multiform glioblastoma	Glioma	X	
757	Brain	Malignant meningioma	Malignant meningioma	Agree	
759	Brain	Oligodendroglioma	Mixed glioma	X	
1216	Brain	Gliosis	Gliosis		Agree
1266	Brain	Multiform glioblastoma	Oligodendroglioma	X	
1426	Brain	Oligodendroglioma	Mixed glioma	X	
1446	Brain	Oligodendroglioma	Glioma	X	
117	Pituitary gland	Adenocarcinoma	Adenocarcinoma	Agree	
117	Brain	Invasion of adenocarcinoma	Invasion of adenocarcinoma	Agree	
1762	Pituitary gland	Adenocarcinoma	Cystic changes	XX	
1762	Brain	Invasion of adenocarcinoma	Metastatic malignant tumor, primary site unknown	X	
1265	Thymus and mediastinal. lymph node	Lymphoblastic lymphoma	Lymphoblastic lymphoma	Agree	
706	Liver	Lymphoblastic leukemia	Lymphoma	X	
1232	Thymus and mediastinal. lymph node	Lymphocytic lymphoma	Lymphocytic lymphoma	Agree	
1209	Lung	Lymphoimmunoblastic lymphoma	Lymphoimmunoblastic lymphoma	Agree	

TABLE 1
Comparison of ERF and NTP PWG Diagnoses of Selected Lesions from the ERF Aspartame Study

Animal #	Tissue	RF Diagnosis	PWG Majority Diagnosis	Agreement	
				Tumor	Non-tumor
212	Thymus and mediastinal. lymph node	Lymphoimmunoblastic lymphoma	Lymphoimmunoblastic lymphoma	Agree	
212	Mesenteric lymph node	Lymphoimmunoblastic lymphoma	Lymphoimmunoblastic lymphoma	Agree	
1449	Thymus and mediastinal. lymph node	Histiocytic sarcoma	Histiocytic sarcoma	Agree	
1449	Liver	Histiocytic sarcoma	Histiocytic sarcoma	Agree	
1451	Liver	Monocytic leukemia	Lymphoma/leukemia	X	
1391	Spleen	Myeloid leukemia	Granulocytic leukemia	± *	
48	Renal pelvis R	Papillary hyperplasia with moderate atypia	Hyperplasia		±
48	Renal pelvis L	Papillary hyperplasia with marked atypia	Hyperplasia		±
51	Renal pelvis R	Hyperplasia; hydronephrosis; pyelitis	Agree		Agree
51	Renal pelvis L	Papilloma with atypia	Papilloma	±	
55	Renal pelvis R	Papillary hyperplasia with marked atypia	Hyperplasia		±
58	Renal pelvis R	Hyperplasia with marked atypia	Hyperplasia		±
58	Renal pelvis L	--	--		NA
73	Renal pelvis R	Hyperplasia with marked atypia	Hyperplasia		±
73	Renal pelvis L	Transitional cell carcinoma	Transitional cell carcinoma	Agree	
176	Renal pelvis R	Early transitional cell carcinoma	Hyperplasia	XX	
176	Renal pelvis L	--	--		NA
1527	Renal pelvis R	Pyelitis	Agree		Agree
1527	Renal pelvis L	Hyperplasia w moderate atypia; pyelitis	Agree		Agree
1571	Renal pelvis L	Papillary hyperplasia with moderate atypia; pyelonephritis	Hyperplasia; pyelonephritis		±
1571	Adrenal gland L	Pheochromocytoma; cortical necrosis	Pheochromocytoma; cystic degeneration	Agree	XX
1700	Renal pelvis R	Early transitional cell carcinoma; pyelonephritis	Hyperplasia; pyelonephritis	XX	Agree
1700	Renal pelvis L	--	Papillary necrosis		XX
1506	Zymbal gland	Squamous cell carcinoma	Squamous cyst	XX	
1506	Lung	Metastasis of squamous cell carcinoma	Squamous cell carcinoma, metastatic, primary site unknown	Agree	
131	Ear duct	Squamous cell carcinoma	Squamous hyperplasia	XX	
205	Ear duct	Squamous cell carcinoma	Agree	Agree	
30	Ear duct	Early squamous cell carcinoma	Squamous hyperplasia	XX	
50	Ear duct	Early squamous cell carcinoma	Squamous hyperplasia	XX	
89	Ear duct	Early squamous cell carcinoma	Agree	Agree	
1614	Ear duct	Early squamous cell carcinoma	Squamous hyperplasia	XX	

TABLE 1
Comparison of ERF and NTP PWG Diagnoses of Selected Lesions from the ERF Aspartame Study

Animal #	Tissue	RF Diagnosis	PWG Majority Diagnosis	Agreement	
				Tumor	Non-tumor
1753	Ear duct	Squamous dysplasia	Squamous hyperplasia 3:2		X
3	Olfactory epithelium	Olfactory neuroblastoma	Olfactory neuroblastoma	Agree	
3	Brain	Invasion of olfactory neuroblastoma	Invasion of olfactory neuroblastoma	Agree	
16	Olfactory epithelium	Hyperplasia	"Not a primary hyperplasia"		XX
23	Olfactory epithelium	Olfactory neuroblastoma	Schwannoma/Sarcoma	X	
29	Olfactory epithelium	Early adenoma	Hyperplasia	XX	
48	Olfactory epithelium	Early adenoma	Glandular hyperplasia	XX	
159	Olfactory epithelium	Hyperplasia	Inflammation		XX
203	Olfactory epithelium	Hyperplasia	Hyperplasia		Agree
215	Olfactory epithelium	Hyperplasia	Hyperplasia		Agree
28	Oral cavity	Early squamous cell carcinoma	Squamous hyperplasia	XX	
123	Oral cavity	Early squamous cell carcinoma	Early squamous cell carcinoma	Agree	
1507	Oral cavity	Early squamous cell carcinoma	Squamous hyperplasia	XX	
29	Forestomach	Squamous cell dysplasia	Squamous hyperplasia		XX
28	Glandular stomach	Adenocarcinoma	Neuroendocrine carcinoma	X	
33	Forestomach	Squamous cell dysplasia	Squamous hyperplasia		XX
33	Pylorus	Adenocarcinoma?	No	XX	
1587	Femur w. lump	Osteosarcoma	Osteosarcoma/sarcoma 3:3	±	
1587	Part of lump	Osteosarcoma	--	NA	

Agree = Complete agreement
± = Partial agreement (e.g., agree on primary diagnosis but disagree on specific detail), or tied PWG vote.
X = Disagreement (e.g., different type of tumor)
XX = Complete disagreement (e.g., tumor v. no tumor)
* Myeloid leukemia and granulocytic leukemia are synonymous.

One reason for some of the disagreement may be the autolysis seen in some of the slides. The PWG report stated that not all of the reviewing pathologists offered a diagnosis in some cases because of "too much autolysis" which can interfere with accurate diagnosis. Indeed, it has been suggested that some of the lung lymphomas may actually represent autolytic adenomas, rather than lymphomas (J.M. Ward, personal communication).

EPA (2009) itself has tacitly acknowledged the mischaracterization of the ERF diagnoses in its comments on another type of tumor claimed by ERF to be associated with methanol exposure.

USEPA (2009) noted: "In their limited review of pathology slides from the ERF aspartame bioassay (2005, 087840; Soffritti et al., 2006, 196735), NTP pathologists interpreted a majority of such head pathologies, including in the ear duct, as being hyperplastic in nature, not carcinogenic (EFSA, 2006, 196098; Hailey, 2004, 089842)."

Moreover, the slides reviewed by the NIEHS PWG represent only a tiny fraction of the thousands of slides that must have been prepared for a study of this size. The original study (Soffritti et al. 2006) reportedly included a total of 1,800 animals; for each animal 40 or more slides would likely have been prepared and examined to generate the overall results. Thus, the 79 slides reviewed by the NIEHS scientists likely represent only about one-tenth of one percent (0.1%) of the slides from the study. Indeed, as noted above, the NIEHS report specifically says that "this review was not considered a 'peer review' of the pathology data from this study" (Hailey 2004).

Not only do the slides reviewed by NTP represent just a tiny fraction of the total slides in the study, they appear to be an unrepresentative sample. For example, while 243 (about 72%) of the animals diagnosed with hemolymphoreticular neoplasms in the ERF study of aspartame had tumors identified in the lung (some of which were also found in other tissues), only one of the ten hemolymphoreticular neoplasms supplied by the ERF for examination by NIEHS was of lung tissue.

Given the substantial disagreement between the ERF diagnoses and those of the NIEHS for this small and unrepresentative sample of the complete set of slides, the NIEHS effort cannot be said to confirm the ERF pathology findings. Indeed, the results from the NIEHS strongly suggest that there are serious diagnostic mischaracterizations in the ERF pathology reports on aspartame. This, in turn, raises the possibility of similar mischaracterizations in the studies of methanol and other compounds studied by ERF.

Indeed, in a recent conversation with the NIEHS PWG Chair, Dr. James R. Hailey, Dr. Hailey made it clear that because of the limited numbers of slides reviewed, and the lack of opportunity for follow-up, he did not consider that the review in any way endorsed the findings of the ERF. While the reviewers were generally aware of the possibility of mycoplasma infection of the animals, the limited review provided no opportunity to investigate whether mycoplasmosis may have caused or confounded the diagnosis of these lesions (Hailey, personal communication, 3/10/2010).

In sum, the results of the NIEHS PWG do not confirm or endorse ERF findings. In fact, the NIEHS results strongly indicate that there are serious diagnostic mischaracterizations in the pathology reports associated with ERF studies.

The Incidence Rate of Hemolymphoreticular Tumors Reported in ERF Studies

The Draft Toxicological Review presents several discussions of what the Agency describes as evidence that hemolymphoreticular tumors are rare in ERF Sprague-Dawley rats. For example:

> "Of the over 200 compounds tested by ERF, 8 have been associated with an increased incidence of hemolymphoreticular tumors in Sprague-Dawley rats, suggesting that it may be a rare and potentially species/strain-specific finding. These eight chemicals are: methanol, formaldehyde, aspartame, MTBE, DIPE, TAME, mancozeb, and toluene. Methanol, formaldehyde, aspartame, and MTBE share a common metabolite, formaldehyde, and DIPE, TAME, methanol and MTBE are all gasoline-oxygenate additives (Caldwell et al., 2008, 196182)."

This might suggest a consistent mechanistic pattern if this statement were accurate. However, it is not. A review of papers published by ERF (that are publicly available in the United States and identifiable using computer literature search services) bioassays revealed information on hemolymphoreticular tumors in Sprague-Dawley rats in studies of just 31 chemicals. Among these 31 (for a few of which there was more than one study), ERF reported increases in hemolymphoreticular tumors in Sprague-Dawley rats dosed with xylenes (Maltoni et al. 1997), sodium hypochlorite (Soffritti et al. 1997), ethanol – female breeders (Soffritti et al. 2002), benzene (Maltoni et al. 1989), ETBE (Maltoni et al. 1999), trichloroethylene (Maltoni et al. 1988), and vinylidene chloride – offspring (Cotti et al. 1988), in addition to the eight compounds identified by EPA. An effect reported with 15 of 31 substances for which data are available (48%) should not, as EPA suggests, be considered to be a "rare and potentially species/strain-specific finding." Of course, because data are not publicly available for the other chemicals that ERF has reportedly studied, it is not possible to determine how rare or common this finding truly is.

The suggestion by EPA that the reported results with methanol, formaldehyde, aspartame, and MTBE reflect a common mode of action involving formaldehyde (a metabolite of all three of the others) is not supportable. The inconsistency in dose-response relationships in males and females for these four chemicals does not support a common mode of action. Table 2 – which lists tumor incidence and percent animals with tumors for the ERF studies of MTBE, methanol, aspartame, and formaldehyde – summarizes these inconsistencies. As shown in that table, there was an apparent dose-related increase in incidence of lymphomas and leukemias in female rats but not in male rats receiving MTBE or aspartame.

In fact, in the case of MTBE, there was an apparent dose-related *decrease* of lymphomas and leukemias in males. In both sexes and at all dose levels the incidence was within the range of historical controls, with the female MTBE controls at the extreme low end of the historical control range, strongly suggesting that there was no causal relationship between MTBE dosing and the observed lymphomas and leukemias.

Table 2 "Lymphomas/Leukemias" in Sprague-Dawley Rats in Ramazzini Foundation Studies of MTBE, Methanol, Aspartame, and Formaldehyde									
		MTBE		**Methanol**		**Aspartame**		**Formaldehyde**	
		Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Control	**Incidence**	10/60	2/60	28/100	13/100	31/150	13/150	8/100	7/100
	%	**16.67%**	**3.33%**	**28.00%**	**13.00%**	**20.67%**	**8.67%**	**8.00%**	**7.00%**
Dose 1	**Incidence**	9/60	6/60	35/100	24/100	23/150	22/150	4/50	5/50
	%	**15%**	**10%**	**35.00%**	**24.00%**	**15.33%**	**14.67%**	**8.00%**	**10.00%**
Dose 2	**Incidence**	7/60	12/60	36/100	24/100	25/150	30/150	10/50	7/50
	%	**11.67%**	**20%**	**36.00%**	**24.00%**	**16.67%**	**20.00%**	**20.00%**	**14.00%**
Dose 3	**Incidence**			40/100	28/100	33/150	28/150	13/50	8/50
	%			**40.00%**	**28.00%**	**22.00%**	**18.67%**	**26.00%**	**16.00%**
Dose 4	**Incidence**					15/100	19/100	12/50	7/50
	%					**15.00%**	**19.00%**	**24.00%**	**14.00%**
Dose 5	**Incidence**					20/100	25/100	11/50	11/50
	%					**20.00%**	**25.00%**	**22.00%**	**22.00%**
Dose 6	**Incidence**					29/100	25/100	23/50	10/50
	%					**29.00%**	**25.00%**	**46.00%**	**20.00%**

With methanol and formaldehyde, there appeared to be increase in lymphomas and leukemias in both sexes, with perhaps a slightly greater effect in males than females – the opposite of the situation with MTBE. This inconsistency argues against a common mechanism of action involving metabolic generation of methanol and formaldehyde from MTBE and aspartame as EPA proposed. Moreover, recently the NTP Expert Panel (2009) for the 12th Report on Carcinogens assessment of formaldehyde dismissed the ERF bioassay of formaldehyde as follows:

> "This finding was not judged to be informative due to the pooling of all types of hemolymphoreticular neoplasias, including those of different cell lineages and the lack of detail provided on the cellular origin and site. Photographic documentation of the hemolymphoreticular neoplasias provided in the 1989 publication raised additional concerns."

Thus, the ERF studies do not reflect a common mode of action involving formaldehyde. Further, comments submitted by the Methanol Institute make a strong case that the common mechanism of action involving metabolic generation of methanol and formaldehyde that EPA

has theorized is not supportable. We refer the reader to those comments for a full discussion of this issue.

Possible Chronic Mycoplasma Infection Confounding Interpretation of Lung Lesions

Perhaps the most critical factor that raises questions regarding the utility of the ERF bioassay of methanol for risk assessment is the likelihood that the animals were suffering from a chronic respiratory infection, probably due to mycoplasma. EPA discounts the substantial circumstantial evidence that has been adduced regarding this problem with ERF studies (Cruzan et al. 2007. 2009; EFSA 2006; ENVIRON 2007, 2008; FDA 2007; McGregor 2006; Schoeb et al. 2009; Ward and Alden 2009 – included as attachments to this submission) by noting that "the existence of an *M. pulmonis* infection in the rat colony used for the ERF methanol study has not been confirmed (Caldwell et al., 2008, 196182)." The Agency is incorrect.

In sworn deposition testimony, Fiorella Belpoggi of ERF acknowledged that antibodies to *M. pulmonis* have been detected in routine veterinary screening of ERF colony rats (relevant pages are attached to this document). While these screened animals may not have been suffering from a frank respiratory disease, the presence of the antibodies is concrete evidence that they were undoubtedly exposed to the microorganism. Moreover, the detection of antibodies indicates that the entire ERF rat colony likely was infected with mycoplasma. Unlike in mice, in which *M. pulmonis* is often rapidly fatal, rats show much less sign of infection, with no adverse effect on their lifespan (Lindsey et al. 1971; Cassell et al. 1973). Caldwell et al. (2008) and USEPA (2009) fail to point out that the lack of more direct evidence of infection may be due to the failure of ERF to perform the appropriate testing that would answer this question definitively, and since the ERF laboratory refuses to allow independent review of their slides and animal colony, no other party is in a position to confirm or deny it.

USEPA (2009) also suggests that if the ERF rat colony was infected, the mycoplasma would have an effect on survival rates. Citing Caldwell et al. (2008) for support that early mortality should be expected, but is not seen in ERF studies (at p 4-116), the Agency concludes that the ERF rat colony is probably not infected. However, Caldwell et al. (2008) rely exclusively on an article by Lindsey et al. (1971) for support that mycoplasma is a cause of early death in rats. The Caldwell et al. (2008) analysis is mistaken. Lindsey et al. (1971) report early mortality only for animals with chronic pulmonary disease (CPD). While Lindsey et al. (1971) also propose that mycoplasma *may* develop into CPD, they provide no evidence or analysis what-so-ever that early mortality should be expected in rats with mycoplasma that has not progressed to CPD. In fact, as cited above, rats with mycoplasma may show much less sign of infection, with no adverse effect on their lifespan (Lindsey et al. 1971; Cassell et al. 1973).

Moreover, the pathology tables released by ERF for the methanol bioassay show clear indicia of infection. For example, inflammation of the lung or bronchus was seen in about 52% of all animals, and inflammation was also commonly seen in the brain (20% of all animals), ear (69% of all animals), and nasal sinuses (21% of all animals), with no indication of a treatment-related effect.

Despite these observations, USEPA (2009) also discounts the possible role of mycoplasma infection in the etiology of the lesions diagnosed by ERF as lymphomas because, it says, "60% of reported lymphoma incidences involved other organ systems, and the dose-response for lymphomas in other organ systems is not remarkably different than for all lymphomas." This statement is profoundly misleading. The only type of lymphoma to show an apparent treatment-related increase in incidence was the type diagnosed by ERF as "lymphoimmunoblastic lymphoma." A total of 171 of these were identified among all groups in the ERF bioassay of methanol (Table 3). Of these, 155 (91%) were identified in the lung, an extremely unusual pattern of occurrence for a lymphoma, which are normally found in lymph nodes or spleen.

Table 3
Animals with "LymphoimmunoblasticLymphoma " (LIL) Diagnosed in the Lung in the Ramazzini Foundation Study of Methanol*

Methanol Concentration in Drinking Water (ppm)	Males		Females	
	Total LIL	Lung LIL	Total LIL	Lung LIL
0	16	16	9	9
500	24	23	17	10
5,000	28	26	19	16
20,000	37	36	21	19

* Data from supplementary table "P04: Neoplasms by Individual Animal" presented on ERF website.

While some of the lymphomas were also found in other tissues in addition to the lung, these were generally lymph nodes and spleen to which tumor cells could easily have metastasized from the lung. Again, the problem is that, because Ramazzini has not been willing to permit a proper peer review of the slides, it is not possible to tell whether and to what extent this issue would affect the results of the study or its interpretation.

In their recent evaluation of ERF bioassays, Schoeb et al. (2009) have noted:

> Questions regarding the role of *M. pulmonis* disease and the histopathologic diagnosis of the lung lesions in the studies in question could be settled by 2 straightforward measures. First, the histologic slides from the studies in question could be subjected to full and independent review by a qualified Pathology Working Group. Examination of

respiratory tract sections in addition to those of lung would be helpful, because suppurative respiratory mucosal inflammation and epithelial hyperplasia and metaplasia would be present in *M. pulmonis* disease but would not be characteristic of lymphoma. Second, tissues from the studies in question, and samples from animals in studies currently in progress, could be tested for *M. pulmonis*. Several methods exist for detection of *M. pulmonis* infection in rodents. For the aspartame, MTBE, methanol, and other completed studies, paraffin-embedded tissues could be tested for *M. pulmonis* by a polymerase chain reaction (PCR) method. Several such methods have been described, one of which we developed and have used successfully on paraffin-embedded tissues.

Until ERF permits either of the above "straightforward measures" to be undertaken with respect to its bioassays, and the potential that infection has confounded the results of those studies can be thoroughly evaluated, ERF studies cannot be relied on as the basis for sound regulatory actions.

Possible Compromised Histopathological Diagnoses Because of Autolysis

The standard ERF study design allows the animals to live until spontaneous death. As a result, if animals die after normal working hours, or they are left unattended after they die, their tissues may become autolized before they are recovered and preserved. This is not a mere theoretical problem. In fact, autolytic changes in tissues were noted in the written summary of a limited review of self-selected slides from an ERF aspartame bioassay that NIEHS pathologists performed (Hailey 2004). The NIEHS reviewers frequently pointed out the difficulty in making diagnoses because of "significant autolytic change" in various tissues.

The draft Methanol Assessment disregards any concern about autolytic changes because according to the Agency, possible losses due to autolysis are "offset by large group sizes." Further, EPA also concludes that "even if autolysis was a confounding factor, its presence would not negate positive cancer findings as autolysis would tend to decrease, not increase, the power to observe an effect." (Draft Toxicological Review at p. 5-46). The truly important concern with autolysis, however, is not that autolytic changes may have resulted in a numerical loss in the number of animals, but rather that the examination of partly autolyzed tissue is likely to have caused erroneous diagnoses. Indeed, as discussed in the following section, some of the lung lymphomas reported by ERF pathologists may actually represent autolytic adenomas, rather than lymphomas (J.M. Ward, personal communication). This observation is consistent with the reported results of several of the NIEHS reviewers who did not offer an opinion on some of the slides reviewed – slides that represented findings of tumors by ERF pathologists – because of "too much autolysis or uncertainty relative to the correct diagnosis" (Hailey (2004). Thus, EPA is clearly incorrect as to whether the presence of autolysis can negate positive cancer findings. Without an independent evaluation of study slides, it is not possible to tell whether and to what extent autolysis may have affected the results of ERF studies or their interpretation.

Variability of Hemolymphoreticular Tumors in Control Groups

The background incidence of hemolymphoreticular tumors in ERF control groups is highly variable, and this variability has at least the potential to affect conclusions drawn from the appearance of such tumors in ERF bioassays. Although EPA seems to recognize this variability, at different points in the IRIS Draft, USEPA (2009) makes conflicting statements regarding the background incidence of hemolymphoreticular tumors reported in control groups of ERF studies. For example, in Section 5.4.3.2 (page 5-47), EPA says:

> "There is also a wide range in the background incidence of hemolymphoreticular tumors reported in control groups of ERF studies."

But a few lines later, EPA says:

> "Caldwell et al. (2008, 196182) noted that for [*sic*] the incidences of these lesions for the ERF colony are relatively low and stable across studies."

In fact, the incidence of hemolymphoreticular tumors in ERF studies is not low; it is highly variable. Table 4 presents a summary of the incidence of "lymphomas and leukemias" in 3,062 male and 3,241 female Sprague-Dawley rats that formed the control groups reported in ERF studies published between 1980 and 2006. As indicated there, the overall average incidence in these studies was 12.2% in males and 7.0% in females, but the range was broad, from 0% (0/90) to 31.8% (35/110) in males, and from 0% (0/50) to 23.3% (14/60) in females. Some information on historical control rates is also presented in Soffritti et al. (2006a). There, a substantially higher incidence is presented. Soffritti et al (2006a) state that among 2,265 untreated males and 2,274 untreated females, the average incidence of "lymphomas/leukemias" was 20.6% (range, 8.0 – 30.9%) in males, and 13.3% (range, 4.0 – 25.0%) in females. The reason for this discrepancy is unclear, but Soffritti et al. (2006a) appear to have omitted the several control groups identified in Table 4 in which no lymphomas or leukemias were reported in the original publications. Whatever the reason, the effect is the same: it undermines confidence in conclusions drawn from the appearance of hemolymphoreticular tumors in exposed animals.

Table 4
"Lymphomas and Leukemias" in Control Groups of Sprague-Dawley Rats in Ramazzini Foundation Studies*

Chemical	Control Male		Control Female		Reference
	Incidence	%	Incidence	%	
MTBE	10/60	16.67%	2/60	3.3%	Belpoggi et al. 1995, 1997; Soffritti et al. 1998
Methanol	28/100	28.00%	13/100	13.00%	Soffritti et al. 2002a
Aspartame	31/150	20.67%	13/150	8.67%	Soffritti et al. 2005, 2006a; Belpoggi et al. 2006a
Formaldehyde	8/100	8.00%	7/100	7.00%	Soffritti et al. 1989, 2002b
Ethanol breeders	35/110	31.82%	17/110	15.45%	Soffritti et al. 2002a
Ethanol offspring	8/49	16.33%	39/277	14.08%	Soffritti et al. 2002a
Coca-Cola breeders	51/235	13.19%	11/55	20.00%	Belpoggi et al. 2006b
Coca-Cola offspring	62/291	21.31%	39/277	14.08%	Belpoggi et al. 2006b
Acetaldehyde	6/50	12.00%	2/50	4.00%	Soffritti et al. 2002b
Mancozeb	16/75	21.3%	11/75	14.67%	Belpoggi et al. 2002a
TAME & DIPE	17/100	17%	7/100	7%	Belpoggi et al. 2002b
ETBE	3/60	5%	3/60	5%	Maltoni et al. 1999
Vinyl acetate breeders	0/14	0.00%	8/37	21.62%	Maltoni et al. 1997a; Minardi et al. 2002
Vinyl acetate offspring	13/107	12.15%	11/99	11.11%	Maltoni et al. 1997a; Minardi et al. 2002
Vinyl chloride breeders			2/60	3.33%	Maltoni & Cotti 1988
Vinyl chloride offspring	12/158	7.59%	1/149	0.67%	Maltoni & Cotti 1988
Vinylidene chloride breeders			2/60	3.33%	Cotti et al. 1988
Vinylidene chloride offspring	12/158	7.59%	1/149	0.67%	Cotti et al. 1988
Styrene (inhal)	3/60	5.00%	3/60	5.00%	Conti et al. 1988
Styrene (gavage)	0/40	0.00%	1/40	2.50%	Conti et al. 1988
Styrene (ip)	3/40	7.50%	0/40	0.00%	Conti et al. 1988
Styrene (sc)	1/40	2.50%	1/40	2.50%	Conti et al. 1988
Styrene oxide (gavage)	2/40	5.00%	1/40	2.50%	Conti et al. 1988
p-Methylstyrene	2/30	6.67%	2/30	6.67%	Conti et al. 1988
p-Methylstyrene	4/60	6.67%	14/60	23.33%	Conti et al. 1988
Propylene (inhal)	2/120	1.67%	4/120	3.33%	Ciliberti et al. 1988
FC11 & FC12	9/150	6.00%	8/150	5.33%	Maltoni et al. 1988
FC22	5/60	8.33%	1/60	1.67%	Maltoni et al. 1988
Trichloroethylene (8 wk)	17/90	18.89%	8/90	8.89%	Maltoni et al. 1988b

Table 4 "Lymphomas and Leukemias" in Control Groups of Sprague-Dawley Rats in Ramazzini Foundation Studies*					
	Control Male		**Control Female**		
Trichloroethylene (104 wk)	9/135	6.67%	7/145	4.83%	Maltoni et al. 1988b
Acrylonitrile (BT 203)	4/75	5.3%	3/75	4%	Maltoni et al. 1988c
Acrylonitrile (BT 201)	0/30	0%	0/30	0%	Maltoni et al. 1988c
Acrylonitrile (breeders)			2/60	3.3%	Maltoni et al. 1988c
Acrylonitrile (offspring)	12/158	7.6%	1/149	0.7%	Maltoni et al. 1988c
Methylene chloride (olive oil)	3/50	6%	1/50	2%	Maltoni et al. 1988d
Methylene chloride (none)	2/20	10%	0/26	0%	Maltoni et al. 1988d
Zeolites (ip)	3/20	15.00%	2/20	10.00%	Maltoni & Minardi 1988
Ethylene dichloride	0/90	0.00%	3/90	3.33%	Maltoni et al. 1980
Benzene (BT 901)	0/30	0.00%	1/30	3.33%	Maltoni et al. 1989
Benzene (BT 902)	3/50	6.00%	1/50	2.00%	Maltoni et al. 1989
Chlorine	4/50	8.00%	0/50	0.00%	Soffritti et al. 1997
Toluene & Xylene	5/50	10.00%	3/50	6.00%	Maltoni et al. 1997b
Toluene & Xylene	3/50	6.00%	1/50	2.00%	Maltoni et al. 1997b
Tamoxifen	14/100	14.00%	9/100	9.00%	Maltoni et al. 1997c
Tamoxifen			13/150	8.67%	Maltoni et al. 1997c
Tamoxifen			12/139	8.63%	Maltoni et al. 1997c
Weighted Mean:		12.2%		7.4%	
* Data are reported only for published studies in which data for lymphoma/leukemia were reported. Some (e.g., the Soffritti et al. (2006b) study of sodium arsenite) did not report lymphoma/leukemia incidence. TAME = *tert*-amyl methyl ether; DIPE = di-isopropyl ether; ETBE = ethyl-*tert*-butyl ether					

Absence of GLP

ERF has never demonstrated its adherence to Good Laboratory Practices (GLP). GLP guidance and regulations were enacted throughout the world by regulatory agencies and other authoritative bodies in response to ethical, and in some cases criminal, lapses by certain testing facilities that jeopardized the reliability of studies intended to support the safety of chemicals and products. Consequently, to ensure reliability, all testing conducted for regulatory approval in the US, Europe, Japan, and other countries must be performed in accordance with GLP regulations. GLP requirements help ensure the quality of the toxicology data, and "reliable and traceable data" (Spindler and Seiler 2002), as well as another very important objective -- transparency (Merz and Wittlinger 1990). GLP requirements (OECD 1997) stress the importance of resources (e.g., qualified personnel); protocols; standard operating procedures; test substance and test system characterization; documentation, including raw data, final report and archives, and a quality assurance unit independent of the individuals performing the study.

Because toxicity studies, especially those involving long-term dosing, are logistically complex and yield enormous amounts of data, the opportunities for error are substantial. GLP requirements are designed to minimize error by ensuring that studies are conducted by experienced scientists and technicians, and that all of the procedures used are fully documented. Highly detailed protocols are required, and any deviations from those protocols that are necessary during the study need to be fully justified and documented. Records involving observations and measurements made during the study are required to be complete in all respects. Animal health must be monitored, and instances of disease outbreaks unrelated to treatment must be recorded. These and many other aspects of study conduct and documentation are typically required with respect to studies submitted to regulatory agencies in connection with chemical product approvals. In developing their evaluations of risk, regulatory agencies now rely heavily on studies that have been conducted under GLP regulations. For example, in the U.S., GLP compliance is required for all studies submitted to FDA (21 CFR 58), or to EPA for studies conducted in support of pesticide regulations (40 CFR 160) or for testing required under Section 4 of the Toxic Substances Control At (TSCA; 40 CFR 792).

The EPA has admitted the existence of several critical issues regarding ERF's compliance with GLP but chooses to minimize their significance. In citing the European Food Safety Authority (EFSA) 2006 review of the ERF bioassay of aspartame, EPA says:

> The EFSA (2006, 196098) report also identifies specific deviations from OECD guidelines (OECD, 2007, 196300), including a lack of a complete analysis of the test substance, no clear information on the stability of the substance, a lack of clinical observations or macroscopic changes, a lack of hematological assays, a lack of serology (e.g., to confirm the presence of infection) and limited histopathology reports. While these details may be recorded internally by the ERF as part of their standard protocol, because there is no documentation of these details available for consideration, there remains some uncertainty regarding the level at which they were performed.
>
> (Draft IRIS Review at p. 5-45).

Notwithstanding the above problems, EPA concludes, "There is limited evidence, however, that these factors had a significant impact on the adequacy of the study for assessing carcinogenic potential." (Draft IRIS Review at p. 5-45). One has to question why the departures from GLP were deemed important by EFSA, but not by US EPA. The Agency's conclusion is inaccurate as the ERF's GLP deficiencies by themselves demonstrate why so many critically important questions remain as to "the adequacy of the study for assessing carcinogenic potential." Those many deficiencies that EPA has acknowledged represent further examples of the lack of clarity and transparency regarding how the ERF study was conducted and how reliable the results are. Moreover, the ERF's limited publication of their data and their refusal to

allow independent review of their findings prevent any reasonable degree of independent confirmation or evaluation of their results.

The Huff Paper Does Not Establish ERF's Compliance with GLP

USEPA (2009) also states that "an independent review of ERF (Huff, 2002, 090326) suggests that quality control procedures associated with GLP were in place." The cited paper by Huff (2002), however, is simply a comparison of results reported by ERF and NTP for the few chemicals that both labs have studied for carcinogenicity. It is not an independent review of the compliance of the ERF laboratory with GLP, and provides no indication of the nature of the "GLP" procedures followed by ERF. In short, ERF has failed to demonstrate adherence with any generally recognized GLP.

Moreover, the actual purpose of the paper by Huff (2002) was to present a comparison between the results of ERF and NTP, and at least in part, attempts to answer whether the differences in responses observed are due to the different protocol employed by ERF. Huff (2002) compared the results of bioassays for the 14 chemicals that have been tested by both ERF and NTP (Table 5). Of those, both laboratories used the same route of administration for 8 chemicals, and they used different routes of exposure for 6 chemicals.

Table 5.
Chemicals Tested in Both the Ramazzini Foundation and NTP Programs (Huff 2002)

	Route of Exposure	
Chemical	**Ramazzini Foundation**	**NTP**
Acrylonitrile	Inhalation, Gavage	Inhalation
Benzene	Inhalation, Gavage	Gavage
Chlorine	Drinking water	Drinking water
Diesel fuel	Gavage	Dermal
Ethylbenzene	Gavage	Inhalation
Methylene chloride	Inhalation, Gavage	Inhalation
Propylene	Inhalation	Inhalation
Styrene	Inhalation, Gavage, Injection	Gavage
Styrene oxide	Gavage	Gavage
Toluene	Gavage	Inhalation
Trichloroethylene	Inhalation	Gavage
Trichlorofluoromethane	Inhalation	Gavage
Vinylidene chloride	Inhalation	Gavage
Xylenes	Gavage	Gavage

In Huff's analysis, results were classified as "positive" if a chemical-related carcinogenic response was reported in one or more target organs, and "negative" if there was no evidence of carcinogenic activity related to chemical exposure. Site concordance was reported if there was a correlation of similar positive responses between laboratories or no evidence of carcinogenic responses from both laboratories. If there was no correlation of responses between laboratories, Huff (2002) reported no site concordance.

For those compounds (xylenes, vinylidene chloride, and toluene) for which there was a difference in classification as positive (showing a carcinogenic response of some type) or negative between NTP and ERF, protocol differences were noted by Huff (2002). In the case of xylenes, while classified as positive by ERF, no tumor incidences were increased until after week 112 of the study, and increases in the incidence of hemolymphorecticular neoplasms were not observed until week 144 of the study. This is much later than the standard study termination of NTP of approximately 104 weeks, and Huff suggested that this may explain the differences in classification, though a tumor that killed an animal at 112 or even 144 weeks might well have been detectable microscopically at 104 weeks. In addition, while increases in several tumor responses were reported by ERF, Huff (2002) noted that none of the increases was dose-related. Therefore, while classified by ERF as "positive," this classification is questionable due to the lack of a dose-related increase in the response.

For vinylidene chloride, increases in total malignant tumors, an endpoint not typically considered by NTP, was part of the basis for a positive classification by ERF, with an increase in the incidence of leukemia also providing support. The incidence of leukemia was noted only in animals with exposure initiating *in utero* and continuing for two years after birth. In the breeding animals, only exposed for two years of their lifespan, no increase in the incidence of this endpoint was noted. Therefore, this difference in protocol, as well as the difference in tumor combinations, contributed to a difference in response between laboratories.

In considering more closely the results for those compounds classified as positive by both laboratories, site concordance for at least one target organ was reported by Huff (2002) for 8 of 11 compounds. These differences between results across laboratories do not appear to be related to route of exposure, since site concordance was observed for selected chemicals with the same or different exposure routes. For two of the eight compounds (chlorine and styrene), Huff (2002) noted that the association of the response with chemical exposure in the NTP study is questionable (Huff 2002). For styrene in particular, Huff (2002) reported a potential site concordance for lung tumors between NTP and ERF, which appears to be an error. The lung tumors reported by NTP were observed in the mouse while ERF did not conduct a bioassay in mice for styrene. While ERF reported an increase in mammary gland tumors in rats exposed to styrene by inhalation, but not gavage, no treatment-related tumors were reported in rats by NTP.

Therefore, this would decrease the number of bioassays with site concordance to seven. Overall, these results suggest that the consistency in response between these two laboratories is only *50%*, with similar responses observed in only 7 of 14 studies conducted using in the same compound.

In sum, the cited paper by Huff is not an independent review of the compliance of the ERF laboratory with GLP. And, in fact, the actual results of the Huff analysis do not indicate that ERF results are consistent with those of NTP.

Inconsistencies in Tumor Counts Reported in Different Versions of the ERF Methanol Study

In addition to the published report describing the ERF bioassay of methanol (Soffritti et al. 2002), tabulations of data from the study have been released on the ERF website. These data tables have changed over time with no explanation. Shown below are pages from two versions of the table identified as "P08: Statistical Analysis of Primary Tumors" for the methanol study. These tables are dated 08/02/2006 and 09/11/2006, respectively, and show the total incidence of hemolymphoreticular tumors in male Sprague-Dawley rats dosed with methanol.

Test Type: CHRONIC-LIFETIME
Route: DOSED WATER
Species/Strain: RATS/SD

P08: STATISTICAL ANALYSIS OF PRIMARY TUMORS
Methyl Alcohol
CAS Number: 67-56-1

Date Report Reqsted: 09/11/2006
Time Report Reqsted: 13:59:59
First Dose M/F: 04/19/90 / 04/19/90
Lab: CRC

STATISTICAL ANALYSIS OF PRIMARY TUMORS IN RATS(SD)
TERMINAL SACRIFICE AT 105 WEEKS

	Males			
DOSE	**20,000 ppm**	**5,000 ppm**	**500 ppm**	**0 ppm**
All Organs				
Malignant Lymphoma: Malignant, Lymphocytic, Lymphoma: Lymphoblastic, Lymphoimmunoblastic, Lymphocytic				
TUMOR RATES	#	#	#	#
OVERALL (a)	38/100 (38%)	29/100 (29%)	27/100 (27%)	17/100 (17%)
POLY-3 RATE (b)	38/80.54	29/75.9	27/79.03	17/68.61
POLY-3 PERCENT (g)	47.2%	38.2%	34.2%	24.8%
TERMINAL (d)	15/42 (36%)	12/38 (32%)	14/42 (33%)	7/36 (19%)
FIRST INCIDENCE	69	342	438	422
STATISTICAL TESTS				
LIFE TABLE	P=0.008**	P=0.072	P=0.197	P=0.009**
POLY 3	P=0.003**	P=0.055	P=0.139	P=0.006**
POLY 1.5	P<0.001**	P=0.043*	P=0.106	P=0.003**
POLY 6	P=0.008**	P=0.074	P=0.173	P=0.013*
LOGISTIC REGRESSION	P<0.001**	P=0.037*	P=0.112	P=0.002**
COCH-ARM / FISHERS	P<0.001**	P=0.032*	P=0.062	P=0.003**
ORDER RESTRICTED	(e)	(e)	(e)	P=0.002**
MAX-ISO-POLY-3	(e)	(e)	(e)	P=0.006**

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TDMS No. 00117 - 68
Test Type: CHRONIC-LIFETIME
Route: DOSED WATER
Species/Strain: RATS/SD

P08: STATISTICAL ANALYSIS OF PRIMARY TUMORS
Methyl Alcohol
CAS Number: 67-56-1
Pathologist: Tibaldi, E.

Date Report Reqsted: 08/02/2006
Time Report Reqsted: 15:52:26
First Dose M/F: 04/19/90 / 04/19/90
Lab: CRC

STATISTICAL ANALYSIS OF PRIMARY TUMORS IN RATS(SD)
TERMINAL SACRIFICE AT 105 WEEKS

	Males			
DOSE	**20,000 ppm**	**5,000 ppm**	**500 ppm**	**0 ppm**
All Organs				
Malignant Lymphoma: Malignant, Lymphocytic, Lymphoma: Lymphoblastic, Lymphoimmunoblastic, Lymphocytic				
TUMOR RATES	#	#	#	#
OVERALL (a)	38/100 (38%)	30/100 (30%)	27/100 (27%)	17/100 (17%)
POLY-3 RATE (b)	38/80.54	30/76.73	27/79.03	17/68.61
POLY-3 PERCENT (g)	47.2%	39.1%	34.2%	24.8%
TERMINAL (d)	15/42 (36%)	12/38 (32%)	14/42 (33%)	7/36 (19%)
FIRST INCIDENCE	69	342	438	422
STATISTICAL TESTS				
LIFE TABLE	P=0.008**	P=0.055	P=0.197	P=0.010**
POLY 3	P=0.003**	P=0.043*	P=0.139	P=0.006**
POLY 1.5	P<0.001**	P=0.032*	P=0.106	P=0.003**
POLY 6	P=0.008**	P=0.061	P=0.173	P=0.014*
LOGISTIC REGRESSION	P<0.001**	P=0.026*	P=0.112	P=0.002**
COCH-ARM / FISHERS	P<0.001**	P=0.022*	P=0.062	P=0.003**
ORDER RESTRICTED	(e)	(e)	(e)	P=0.002**
MAX-ISO-POLY-3	(e)	(e)	(e)	P=0.007**

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As can be seen above, these two versions of what should be the same table show a different incidence of tumors in the 5,000 ppm males (29/100 v. 30/100). While this difference is not large, it adds further to the plethora of questions that are raised regarding the accuracy and reliability of the study and its interpretation. Similar discrepancies exist in different versions of reports of other ERF studies. For example, Table 6 shows different versions of the incidence of hemolymphoreticular tumors in male Sprague-Dawley rats dosed with MTBE.

Table 6 Incidence of Lymphoma and Leukemia Reported in Ramazzini Foundation MTBE Study			
Dose	**Number of Animals with Lymphoma/Leukemia**		
	Belpoggi et al. (1995)[a]	**Belpoggi et al. (1998)**	**Data Tables (2007)[b]**
Males			
Control	10	--[c]	7
Low	9	--	7
High	7	--	6
Females			
Control	2	2	2
Low	6	7	7
High	12	12	12

[a] The same data are presented in Belpoggi et al. (1997).
[b] From data tables (dated 12/13/2007) released on ERF website
[c] Not reported in Belpoggi et al. (1998).

CONCLUSIONS

Several of the criticisms discussed in these comments have the potential to undermine the ERF bioassay of methanol entirely. Moreover, when viewed together, they raise such substantial uncertainties regarding the reliability of the results that it is not scientifically appropriate to rely on them without first completing the following actions:

(i) obtain full study documentation of the type available for NTP studies;
(ii) evaluate the studies using NTP criteria;
(iii) give little weight to any study for which adequate documentation of conduct and results is absent or for which documentation is inconsistent; and
(iv) thoroughly evaluate the role of infection in tumor production in the SD rats used by the ERF, preferably including histopathology peer review by pathologists experienced in diagnosing mycoplasmosis and, if possible, to perform PCR analysis of retained lung tissue samples to identify mycoplasma.

Unless these actions are accomplished, we believe EPA's reliance on these results for assessing the quantitative carcinogenic potency of methanol would be scientifically unsupportable and contrary to the public interest.

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