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PESTICIDE ASSESSMENT GUIDELINES - SUBDIVISION F HAZARD EVALUATION: HUMAN AND DOMESTIC ANIMALS ADDENDUM 10 - NEUROTOXICITY SERIES 81, 82, AND 83

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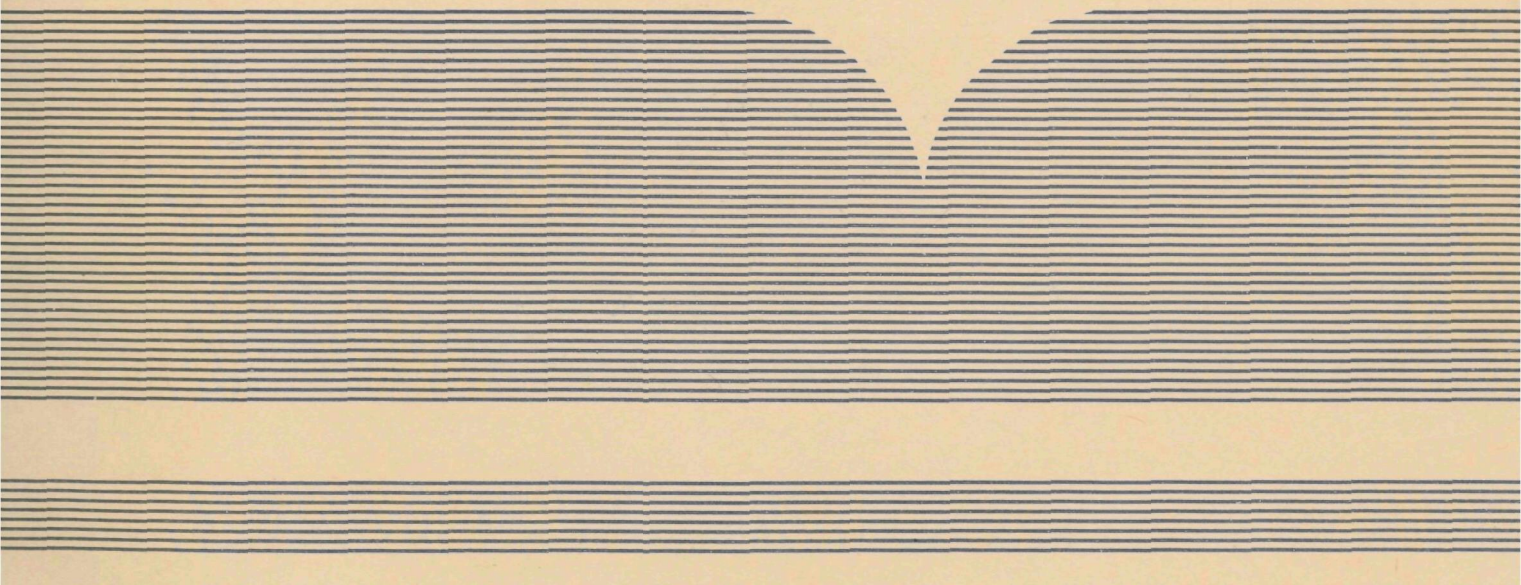

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PESTICIDE ASSESSMENT GUIDELINES

SUBDIVISION F

HAZARD EVALUATION:

HUMAN AND DOMESTIC ANIMALS

ADDENDUM 10

NEUROTOXICITY

SERIES 81, 82, AND 83

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FOREWORD

These new and revised neurotoxicity guidelines are intended to replace and supplement the set of neurotoxicity guidelines originally published in the 1982 Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals; EPA-540/9-82-05; October 1982; National Technical Information Service, Springfield, VA 22161. These guidelines have been written in coordination with upcoming proposed revisions to the Toxicology Data Requirements of Part 158 of Title 40 of the Code of Federal Regulations (40 CFR 158). These guidelines have undergone extensive review within the Agency, public comment, and review by the FIFRA Scientific Advisory Panel.

ACKNOWLEDGMENTS

The development and revision of these guidelines was due to the dedicated efforts of many people inside and outside EPA, but predominantly the members of 2 workgroups. Their efforts are here noted and gratefully acknowledged.

The following people participated in the revision of these guidelines as members of the ad hoc Workgroup :Kevin Crofton, Karl Jensen, Tina Levine, Robert MacPhail, Suzanne McMaster, Virginia Moser, Stephanie Padilla, D. Cooper Rees, Lawrence Reiter, Hugh Tilson, and William Sette, chairman.

The Developmental Neurotoxicity Guideline Workgroup included: Zoltan Annau, Angela Auletta, Marlissa Campbell, Neil Chernoff, Kevin Crofton, Lynda Erinoff, Earl Grey, Karl Jensen, Carole Kimmel, Tina Levine, Robert MacPhail, Suzanne McMaster, C.J. Nelson, Myron Ottley, D. Cooper Rees, Larry Reiter, Jennifer Seed, William Sette, Mark Stanton, Hugh Tilson, Hal Zenick, and Elaine Francis as chairwoman.

Subdivision F

Neurotoxicity

Table of Contents

DELAYED NEUROTOXICITY
OF ORGANOPHOSPHORUS SUBSTANCES
FOLLOWING ACUTE AND 28 DAY EXPOSURES

(a) [1]Purpose. In the assessment of organophosphorus substances[1], studies of delayed neurotoxicity using the adult hen as the test animal and including behavioral observation of gait, histopathological assessment of brain, peripheral nerve, and spinal cord, and neurochemical assessment of inhibition of acetylcholinesterase(AchE) and neurotoxic esterase (NTE) are needed to identify and characterize these potential effects.

This guideline now requires an acute dosing regimen in hens in combination with assays of neurotoxic esterase (and acetylcholinesterase) to screen for this effect. Use of data on the inhibition of NTE in conjunction with behavioral and pathological data offers a number of advantages[2]. It is important to recognize that many acute studies can provide equivocal evidence of behavioral or pathological effects. Some trixylenyl phosphates (Johnson, 1975), for example, are negative, or at best equivocal, after acute exposures, yet clearly cause OPIDN after repeated exposures. The continuous, rather than descriptive, nature of NTE data and the fact that considerable inhibition is generally required to produce OPIDN, will help to more convincingly conclude that a substance is negative, based solely on an acute study. Conversely, NTE data can also provide a better indication of potential delayed neurotoxicity, i.e. if the behavioral and histopathological data after an acute exposure are equivocal, and the NTE inhibition is significant, then further study is appropriate.

The revision of the 90 day study to a 28 day study is based on the idea that these shorter exposures offer savings in animals and cost and because 28 days are often closer to the duration of exposure applicators may experience than 90 days. In some cases, further study may be required to resolve data that are difficult to interpret clearly, or to establish more refined dose response relations, or to assess the particular use patterns of the substance.

(b) Definitions. (1) Organophosphorus induced delayed neurotoxicity (OPIDN) is a neurological syndrome in which limb weakness and upper motor neuron spasticity are the predominant clinical signs; distal axonopathy of peripheral nerve and spinal cord are the correlative pathological signs; and inhibtion and aging of neurotoxic esterase in neural tissues are the correlative biochemical effects. Clinical signs and pathology first appear between 1 and 2 weeks following exposures that typically inhibit and subsequently age neurotoxic esterase.

(2) Neuropathy target esterase (NTE) or neurotoxic esterase is a membrane-bound protein that hydrolyzes phenyl valerate. The inhibition and "aging" of the phosphorylated NTE, i.e., the covalent binding of the OP to the enzyme, is highly correlated with the initiation of OPIDN. Not all O-Ps that inhibit

Delayed Neuro

NTE cause OPIDN, but all O-Ps that cause OPIDN inhibit NTE.

(3) NTE activity is operationally defined as the phenyl valerate hydrolytic activity resistant to paraoxon but sensitive to mipafox or neuropathic O-P ester inhibition.

(c) Principle of the test method. The test sequence consists of acute and 28 day exposure studies. Any significant effects on behavior(delayed effects), histopathology, or inhibition of NTE in the acute study are sufficient cause to conduct the 28 day study. The test substance is administered orally to domestic hens that in some cases have been protected from acute cholinergic effects. The animals are observed for at least 21 days for gait changes and other signs. Neurochemical examination of selected neural tissues is undertaken on some animals at some time(s) after exposure. Histopathology of brain, spinal cord, and peripheral nerve are performed at the termination of 21 day observation periods.
If the results of the acute study are completely negative, that is, there are no delayed behavioral effects, and no histopathological effects, and no significant NTE inhibition, then the 28 day study is not required. Otherwise, the 28 day study should be conducted. In the 28 day study, 3 exposure levels are used to describe the dose response curve sufficiently to estimate a reference dose.

(d) Test Procedures. (1) Animal selection. The adult domestic laying hen (Gallus gallus domesticus), aged 8 to 14 months, is recommended. Standard size breeds and strains should be employed. Healthy young adult hens free from interfering viral diseases and medication and without abnormalities of gait should be acclimatized to the laboratory conditions for at least 5 days prior to randomization and assignment to treatment and control groups.

(2) Housing and feeding conditions. Cages or enclosures which are large enough to permit free mobility of the hens and easy observation of gait should be used. Where the lighting is artificial, the sequence should be 12 hours light, 12 hours dark. Appropriate diets should be administered as well as an unlimited supply of drinking water. The hens should be weighed weekly. Any moribund hens should be removed and sacrificed.

(3) Route of Administration. Dosage of test substance should normally be by the oral route, preferably by gavage. Liquids may be given neat or dissolved in an appropriate vehicle such as corn oil; solids should be dissolved if at all possible since large doses of solids in gelatin capsules may significantly impair absorption. Dermal exposures may be the most significant route of exposure for applicators and for non-food uses and there may be important differences in toxicity by this route. Conduct of these studies by this route may be appropriate and should be considered.

(4) Study Design. (i) General. An important consideration for the design of these studies is prediction of activity based on the structure of the material and the published literature. Some materials, e.g. phosphinates, are known to inhibit

NTE, but not to be capable of aging and, thus, are not expected to cause OPIDN. Many materials have structural features that will permit inhibition and aging, i.e. ester linkages, and are of potential concern. Published data are available for many materials and may be very useful for many aspects of the design and interpretation of these studies.

(ii) Dose levels and selection. For the acute study, a single exposure group is required. The acute dose level should be chosen to maximize the amount of material given to the hens, particularly in cases where some activity is expected. For the 28 day study, at least 3 exposure groups are required in addition to the vehicle control group. Ideally, the data should be sufficient to produce a dose-effect curve. We strongly encourage the use of equally spaced doses and a rationale for dose selection that will maximally support detection of dose-effect relations. The rationale for dose selection chosen by the investigator should be explicitly stated. The following guidance for dose selection is somewhat complex and is not intended to be rigidly followed.

(A) Acute Study. Selection of the dose level for the acute study may be based on a limit dose, or lethal doses and other available data, e.g.,on NTE inhibition.

(1) Levels of test substances greater than 2 g/kg need not be tested.

(2) Lethal Doses. Either an LD50 or an approximate lethal dose (ALD) in the hen may be used to determine the acute high dose. If a hen LD50 has been established, then this, of course, may be used, although some verification may be prudent. If the rat LD50 is known, it may serve as the starting point of estimation. A preliminary lethality study in unprotected hens may be conducted to estimate the acute high dose. A variety of test methodologies may be used to estimate the unprotected lethal dose of test materials. Of course, the method of estimate of the lethal dose may influence the subsequent dosage estimates.
From the preliminary data, if cholinergic signs were seen very soon after dosing, prophylaxis using atropine may be appropriate. Atropine (20 mg/kg, s.c., up to every 2 hours) should be used to prevent death from acute cholinergic effects.

(B) 28 Day Study. (1) Levels of test substances greater than 1 g/kg need not be tested.

(2) High dose. The high dose selected should be estimated to be sufficient to cause OPIDN or be a maximum tolerated dose based on the acute data, but not result in an incidence of fatalities that would prevent a meaningful evaluation of the data.

(3) Low dose. The low dose should be estimated to be a minimum effect level, e.g., an ED10, or alternatively, a no effect level.

(4) The intermediate dose level should be equally spaced between the high and low doses.

(5) Intermediate responses in NTE i.e.,

greater than 15% and less than 70%, can be crudely extrapolated as if the dose-response were a simple first order relationship. That is, if a certain dose caused 50% inhibition, then twice that dose might cause 75%. Such extrapolation is very crude but can be useful in giving some guidance for dose estimation.

(iii) Numbers of animals. Exposure groups should be large enough to provide six survivors for both behavioral observations and histopathology. At least 3 hens are required for determination of NTE in each dose or control group and at each time point.

(iv) Control Groups. A positive control group of at least six hens treated with a known delayed neurotoxicant, such as Triorthocresyl phosphate (TOCP), is required for both acute and 28 day studies. This group may be a concurrent or historical control group.(This should also include at least 3 hens assessed for biochemical measurements.) Periodic re-determinations of the sensitivity of the assays is suggested, for historical control data, i.e., when some essential element of the test conduct by the performing laboratory has changed. A concurrent control group sufficient to provide 6 survivors for histopathology and 3 hens for NTE measurement are treated in a manner identical to the treated groups, except that administration of the test substance is omitted. When protective agents are used, all members of the dose groups and vehicle controls should receive the same treatment.

(5) Study Conduct. (i) Biochemical measurements. (A) NTE Assay. The test method is a differential assay of the ability of neural tissue, following O-P exposure, to selectively hydrolyze a phenyl valerate substrate. The principle of the assay is: first, to determine the amount of hydrolysis that occurs in the presence of a non-neurotoxic inhibitor, paraoxon, (a), which is intended to occupy irrelevant sites; Second, to determine the activity in the presence of paraoxon and a known neuropathic inhibitor, mipafox, (b). NTE activity is the difference between (a) and (b), that is, the proportion of activity inhibited only by mipafox. Thus, the "mipafox site" is already occupied following exposure to a neuropathic O-P ester and the activity of (b) is therefore reduced.

(1) Three hens from each group should be sacrificed at 48 hours after the last dose. Depending on the duration of acute signs as an indication of the disposition of the test material, the time for sacrifice for NTE and AchE assessment may be chosen at a different time to optimize detection of effects. Both the brain and spinal cord should be prepared for assay of NTE. Perform duplicate assays of NTE in brain and spinal cord of three birds from each group and control group.

(2) Materials. This assay requires paraoxon (diethyl 4-nitrophenyl phosphate), mipafox (N, N'-diisopropylphosphorodiamido fluoridate), and phenyl valerate. They all can be obtained commercially.

(3) The assay has four stages: Preparation of tissue; differential preincubation; hydrolysis of

substrate; and measurement of product. The quotations that follow are from Johnson (1977) as corrected or modified in Johnson (1982). His is the best known method for conduct of this assay. Other acceptable methods have been used. They primarily involve minor technical modification (Sprague et al. 1981; Soliman et al. 1982).

(a) Preparation of tissue. "the whole brain (is) removed and cooled in ice-cold buffer (50 mM Tris/0.2 mM EDTA adjusted to pH 8.0 at 25° with HCL). Meninges and blood vessels are rapidly removed and the brain is blotted dry, weighed, and homogenized thoroughly in ice-cold buffer(at a volume of at least 1:30, w/v), using a high-speed rotating perspex pestle with not more than 0.25 mm difference in diameter between pestle and tube."

(b) Differential preincubation. "Paired samples of homogenate (equivalent to about 6.0 mg tissue) are pre-incubated in Tris/EDTA buffer pH 8 at 37° for exactly 20 minutes with paraoxon (40 to 100 uM) plus either (a) buffer or (b) mipafox (50uM) in a final volume of 2 ml."

(c) Hydrolysis of substrate. "After preincubation, dispersion (2ml) of phenyl valerate is added and the incubation is continued for exactly 15 minutes. The dispersion is prepared by adding a solution of Triton X-100 (0.03 percent in water) (30 vol) to a solution of phenyl valerate (15 or 20 mg/ml) in redistilled dimethylformamide (1 vol) and mixing thoroughly (by swirling): other solvents give less satisfactory dispersions. Reaction is stopped by adding 2 ml of sodium dodecyl sulphate (1-2% w/v) in buffer containing 4-aminoantipyrine (otherwise known as 4-aminophenazone) (0.25 percent)."

(d) Measurement of product. This assay is based on the colorimetric determination of liberated phenol.

(1) "The coupling of phenol liberated in the assay with the aminoantipyrine may be performed at any convenient time after quenching the enzyme: 1 ml of $K_3Fe(CN)_6$ (0.4 percent in water) is added and the stable red colour is read at 490 nm."

(2) "A nontissue blank, kept to 10 percent of the paraoxon tube value by maintaining the substrate phenol fee, should be included in each group of assay tubes. Typical control absorbance values would be 0.8 for paraoxon, 0.35 for paraoxon and mipafox and 0.07 for the blank. Colour development takes (1-2 min) in solutions stopped with sodium dodecyl sulphate. The extinction coefficient of phenol under these conditions is 15,600 at a wavelength of 490 nm. NTE activity is represented by the difference in absorbance obtained from samples incubated under conditions (a) and (b) respectively."

(3) "Under standard conditions NTE hydrolyzes about 2400 nanomoles of substrate/min/g of cortex, 550 for spinal cord, and 100 for sciatic nerve."

(B) AChE measures. Assay of

acetylcholinesterase in the brains of the same birds (Johnson and Russell, 1975; or Ellman et al. , 1961) shall also be performed. The level of AChE inhibition is a useful index of lethal potency and the ratio of lethal potency to NTE inhibitory potency can be useful for subsequent dose selection.

(ii) 21 Day observation. All remaining hens should be carefully observed at least once daily for a period of at least 21 days and signs of toxicity recorded, including the time of onset, degree, and duration. Observations should include, but not be limited to, behavioral abnormality, locomotor ataxia, and paralysis. At least twice a week the hens should be taken outside the cages and subjected to a period of forced motor activity, such as ladder climbing, in order to enhance the observation of minimal responses. A rating scale of at least four levels should be used to grade ataxia, e.g. Roberts et al. (1983).

(iii) Necropsy and Histopathology. Gross necropsies are recommended for all survivors and should include observation of the appearance of the brain and spinal cord. All animals shall be prepared for microscopic examination. Tissues shall be fixed by whole body perfusion, with a fixative appropriate for the embedding media. Sections should include medulla oblongata, spinal cord, and peripheral nerves. The spinal cord sections should be taken from the rostral cervical, the midthoracic, and the lumbo-sacral regions. Section of the proximal regions of both of the tibial nerves and their branches should be taken. Sections should be stained with appropriate myelin and axon-specific stains.
For 28 day studies, a stepwise examination of tissue samples is recommended. In such a stepwise examination, sections from the high dose group are first compared with those of the control group. If no neuropathological alterations are observed in samples from the high dose group, subsequent analysis is not required. If neuropathological alterations are observed in samples from the high dose group, samples from the intermediate and low dose groups are then examined sequentially.

(e) Data reporting and evaluation. (1) Test report. In addition to any other applicable reporting requirements, the final test report must include the following information:

(i) Toxic response data by group with a description of clinical signs; the criteria for the grading system for ataxia and any other scales should be defined.

(ii) For each animal, time of death during the study or whether it survived to termination.

(iii) The day of the first occurrence of each abnormal sign and its subsequent course including its degree.

(iv) Body weight data.

(v) Necropsy findings for each animal, including a description of the appearance of the brain and the spinal cord.

(vi) Biochemical data for each animal assessed, including absorbance values for each animal tested, and blank

sample data.

(vii) A detailed description of all histopathological findings.

(viii) Statistical treatment of results, where appropriate.

(2) Treatment of results. Data may be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions or effects, the types of lesions or effects and the percentage of animals displaying each type of lesion or effect.

(3) Evaluation of results. The findings of these delayed neurotoxicity studies should be evaluated in terms of the incidence and severity of behavioral, neurochemical, and histopathological effects and of any other observed effects in the treated and control groups, as well as any information known or available to the authors, such as published studies. For a variety of results seen, further studies may be necessary to characterize these effects.

(f) References. For additional background information on this test guideline the following references should be consulted:

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Delayed Neuro

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Delayed Neuro

TABLE 1
STUDY DESIGN

	# OF HENS	NTE/TIME	BEHAVIOR/PATHOLOGY
ACUTE STUDY		HOURS	
POSITIVE CONTROLS	9	3/48	6
VEHICLE CONTROLS	9	3/48	6
DOSE	9	3/48	6
28 DAY STUDY		HOURS	
POSITIVE CONTROLS	9	3/48	6
VEHICLE CONTROLS	9	3/48	6
HIGH DOSE	9	3/48	6
LOW DOSE	9	3/48	6
MID DOSE	9	3/48	6

NOTES

1. Substances appropriate for testing are uncharged esters, thioesters, or anhydrides of organophosphoric, organophosphonic, or organophosphoramidic acids or of the related phosphorothioic, phosphonothioic, or phosphorthioamidic acids.

2. Two commenters questioned the validity or usefulness of NTE measurements.

The Agency believes that this assay will be both valid and useful and that this is the consensus of the scientific community . Only 2 of roughly 30 commenters questioned the addition of this assay. The SAP clearly endorsed this approach. The published literature amply demonstrates that inhibition of this protein is necessary but not sufficient for the initiation of OPIDN, and that it is highly correlated with the other signs of OPIDN, i.e. gait changes, and central-peripheral distal axonopathy. The assay itself was reviewed by the SAP in 1987 and has since been reviewed again after minor revision both in ORD and by a number of reviewers as well as the SAP. We believe that this assay possesses therefore, concurrent, predictive, and content validity. While inhibition of NTE is not, per se, an adverse effect, it is not being used as the sole basis of such assertions.

NEUROTOXICITY SCREENING BATTERY[1]

(a) Purpose. In the assessment and evaluation of the potential human health effects of chemical substances, it is appropriate to test for neurotoxic effects. This neurotoxicity screening battery consists of a functional observational battery, motor activity, and neuropathology. The functional observational battery consists of non-invasive procedures designed to detect gross functional deficits in animals and to better quantify behavioral or neurological effects detected in other studies. The motor activity test uses an automated device that measures the level of activity of an individual animal[2]. The neuropathological techniques are designed to provide data to detect and characterize histopathological changes in the central and peripheral nervous system. This battery is designed to be used in conjunction with general toxicity studies and changes should be evaluated in the context of both the concordance between functional neurological and neuropatholgical effects, and with respect to any other toxicological effects seen This test battery is not intended to provide a complete evaluation of neurotoxicity, and additional functional and morphological evaluation may be necessary to assess completely the neurotoxic potential of a chemical.

(b) Definitions.

(1) Neurotoxicity is any adverse effect on the structure or function of the nervous system related to exposure to a chemical substance.

(2) A toxic effect is an adverse change in the structure or function of an experimental animal as a result of exposure to a chemical substance.

(3) Motor activity is any movement of the experimental animal.

(c) Principle of the test method. The test substance is administered to several groups of experimental animals, one dose being used per group. The animals are observed under carefully standardized conditions with sufficient frequency to ensure the detection and quantification of behavioral and/or neurologic abnormalities, if present. Various functions that could be affected by neurotoxicants are assessed during each observation period. Measurements of motor activity of individual animals are made in an automated device. The animals are perfused and tissue samples from the nervous system are prepared for microscopic examination. The exposure levels at which significant neurotoxic effects are produced are compared to one another and to those levels that produce other toxic effects.

(d) Test procedures. (1) Animal selection. (i) Species. In general, the laboratory rat should be used. Under some circumstances, other species, such as the mouse or the dog, may be more appropriate, although not all of the battery may be

adaptable to other species.

(ii) Age. Young adults (at least 42 days old for rats) shall be used.

(iii) Sex. (A) Both males and females shall be used.

(B) Females shall be nulliparous and nonpregnant.

(2) Number of animals. At least ten males and ten females shall be used in each dose and control group for behavioral testing[3]. At least five males and five females shall be used in each dose and control group for terminal neuropathology. If interim neuropathological evaluations are planned, the number shall be increased by the number of animals scheduled to be perfused before the end of the study. Animals shall be randomly assigned to treatment and control groups.

(3) Control groups. (i) A concurrent (vehicle) control group is required. Subjects shall be treated in the same way as for an exposure group except that administration of the test substance is omitted. If the vehicle used has known or potential toxic properties, both untreated or saline treated and vehicle control groups are required.

(ii) Positive control data from the laboratory performing the testing shall provide evidence of the ability of the observational methods used to detect major neurotoxic endpoints including limb weakness or paralysis (e.g., repeated exposure to acrylamide),tremor (e.g., pp'DDT), and autonomic signs (e.g.,carbaryl). Positive control data are also required to demonstrate the sensitivity and reliability of the activity-measuring device and testing procedures. These data should demonstrate the ability to detect chemically induced increases and decreases in activity. Positive control groups exhibiting central nervous system pathology and peripheral nervous system pathology are also required. Separate groups for peripheral and central neuropathology are acceptable (e.g., acrylamide and trimethyl tin). Positive control data shall be collected at the time of the test study unless the laboratory can demonstrate the adequacy of historical data for this purpose, i.e., by the approach outlined in this guideline[4].

(4) Dose level and dose selection[5]. At least 3 doses shall be used in addition to the vehicle control group. Ideally, the data should be sufficient to produce a dose-effect curve. We strongly encourage the use of equally spaced doses and a rationale for dose selection that will maximally support detection of dose-effect relations.

For acute studies, dose selection may be made relative to the establishment of a benchmark dose[6] (BD). That is, doses may be specified as successive fractions, e.g. 1/2, 1/4, of the BD. The BD itself may be estimated as the highest non-lethal dose as determined in a preliminary range-finding lethality study. A variety of test methodologies may be used for this purpose, and

the method chosen may influence subsequent dose selection. The goal is to use a dose level that is sufficient to be judged a limit dose, or clearly toxic.

(i) Acute Studies. The high dose need not be greater than 2 g/Kg. Otherwise, the high dose shall result in significant neurotoxic effects or other clearly toxic effects, but not result in an indicence of fatalities that would preclude a meaningful evaluation of the data. This dose may be estimated by a benchmark dose procedure as described above, with the middle and low dose levels chosen as fractions of the benchmark dose. The lowest dose shall produce minimal effect, e.g. an ED10, or alternatively, no effects.

(ii) Subchronic (and Chronic) Studies. The high dose need not be greater than 1g/Kg. Otherwise, the high dose level shall result in significant neurotoxic effects or other clearly toxic effects, but not produce an incidence of fatalities that would prevent a meaningful evaluation of the data. The middle and low doses should be fractions of the high dose. The lowest dose shall produce minimal effects, e.g. an ED10, or alternatively, no effects.

(5) Route of exposure. Selection of route may be based on several criteria including, the most likely route of human exposure, bioavailability, the likelihood of observing effects, practical difficulties, and the likelihood of producing non-specific effects. For many materials, it should be recognized that more than one route of exposure may be important and that these criteria may conflict with one another. In order to save resources, initially only one route is being required for screening for neurotoxicity. The route that best meets these criteria should be selected. Dietary feeding will generally be acceptable for repeated exposures studies.

(6) Combined protocol. The tests described in this screening battery may be combined with any other toxicity study, as long as none of the requirements of either are violated by the combination.

(7) Study conduct. (i) Time of testing. All animals shall be weighed on each test day and at least weekly during the exposure period.

(A) Acute Studies. At a minimum, for acute studies observations and activity testing shall be made before the initiation of exposure, at the estimated time of peak effect within 8 hours of dosing, and at 7 and 14 days after dosing. Estimation of time(s) of peak effect may be made by dosing pairs of rats across a range of doses and making regular observations of gait and arousal[7].

(B) Subchronic (and Chronic) Studies[8]. In a subchronic study, at a minimum, observations and activity measurements shall be made before the initiation of exposure and, before the daily exposure, or for feeding studies at the same time of day, during the 4th, 8th, and 13th weeks of exposure. In

chronic studies, at a minimum, observations and activity measurements shall be made before the initiation of exposure and before the daily exposure, or for feeding studies at the same time of day, every 3 months.

(ii) Functional Observational Battery. (A) General Conduct. All animals in a given study shall be observed carefully by trained observers who are unaware of the animal's treatment, using standardized procedures to minimize observer variability. Where possible, it is advisable that the same observer be used to evaluate the animals in a given study. If this is not possible, some demonstration of inter-observer reliability is required. The animals shall be removed from the home cage to a standard arena for observation. Effort should be made to ensure that variations in the test conditions are minimal and are not systematically related to treatment. Among the variables that can affect behavior are sound level, temperature, humidity, lighting, odors, time of day[9], and environmental distractions. Explicit, operationally defined scales for each measure of the battery are to be used. The development of objective quantitative measures of the observational end-points specified is encouraged. Examples of observational procedures using defined protocols may be found in Irwin (1968), Gad (1982), and Moser et al. (1988). The functional observational battery shall include a thorough description of the subject's appearance, behavior, and functional integrity. This shall be assessed through: observations in the home cage; while the rat is moving freely in an open field; and through manipulative tests. Testing should proceed from the least to the most interactive with the subject. Scoring criteria, or explicitly defined scales, shall be developed for those measures which involve subjective ranking.

(B) List of measures. The functional observational battery shall include the following list of measures.

(1) Assessment of signs of autonomic function, including but not limited to:

a) ranking of the degree of lacrimation and salivation, with a range of severity scores from none to severe;

b) presence or absence of piloerection and exophthalmus;

c) ranking or count of urination and defecation, including polyuria and diarrhea. This is most easily conducted during the open field assessment.

d) pupillary function such as constriction of the pupil in response to light or a measure of pupil size;

e) degree of palpebral closure, e.g., ptosis.

(2) Description, incidence, and severity of any convulsions, tremors, or abnormal motor movements, both in

the home cage and the open field.

(3) Ranking of the subject's reactivity to general stimuli such as removal from the cage or handling, with a range of severity scores from no reaction to hyperreactivity.

(4) Ranking of the subject's arousal level or state of alertness during observations of the unperturbed subject in the open field, with a range of severity scores from coma to hyperalertness[10].

(5) Descriptions and incidence of posture and gait abnormalities observed in the home cage and open field.

(6) Ranking of any gait abnormalities, with a range of severity scores from none to severe.

(7) Forelimb and hindlimb grip strength measured using an objective procedure, e.g. that described by Meyer et al. (1979).

(8) Quantitative measure of landing foot splay[11]; the procedure described by Edwards and Parker (1977) is recommended.

(9) Sensorimotor responses to stimuli of different modalities will be used to detect gross sensory deficits. Pain perception may be assessed by a ranking or measure of the reaction to a tail-pinch, tail-flick, or hot-plate. The response to a sudden sound, e.g., click or snap, may be used to assess audition.

(10) Body weight.

(11) Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

(C) Additional measures. Other measures may also be included and the development and validation of new tests is encouraged. Further information on the neurobehavioral integrity of the subject may be provided by:

(1) Count of rearing activity on the open field;

(2) Ranking of righting ability;

(3) Body temperature;

(4) Excessive or spontaneous vocalizations;

(5) Alterations in rate and ease of respiration, e.g., rales or dyspnea;

(6) Sensorimotor responses to visual or proprioceptive stimuli.

(iii) Motor activity. Motor activity shall be monitored by an automated activity recording apparatus. The device used must be capable of detecting both increases and

decreases in activity, i.e., baseline activity as measured by the device must not be so low as to preclude detection of decreases nor so high as to preclude detection of increases in activity. Each device shall be tested by standard procedures to ensure, to the extent possible, reliability of operation across devices and across days for any one device. In addition, treatment groups must be balanced across devices. Each animal shall be tested individually. The test session shall be long enough for motor activity to approach asymptotic levels by the last 20 percent of the session for non-treated control animals. All sessions shall have the same duration. Treatment groups shall be counter-balanced across test times (See endnote 6). Effort should be made to ensure that variations in the test conditions are minimal and are not systematically related to treatment. Among the variables which can affect motor activity are sound level, size and shape of the test cage, temperature, relative humidity, lighting conditions, odors, use of the home cage or a novel test cage, and environmental distractions.

(iv) Neuropathology: Collection, Processing and Examination of Tissue Samples.[12] To provide for adequate sampling as well as optimal preservation of cellular integrity for the detection of neuropathological alterations, tissue shall be prepared for histological analysis using in situ perfusion and paraffin and/or plastic embedding procedures. Paraffin embedding is acceptable for tissue samples from the central nervous system. Plastic embedding of tissue samples from the central nervous system is encouraged, when feasible. Plastic embedding is required for tissue samples from the peripheral nervous system[13].

Subject to professional judgment and the type of neuropathological alterations observed, it is recommended that additional methods, such as Bodian's or Bielchowsky's silver methods, and/or GFAP immunohistochemistry be used in conjunction with more standard stains to determine the lowest dose level at which neuropathological alterations are observed. When such special stains indicate evidence of structural alterations it is recommended that the GFAP radioimmunoassay also be performed, particularly when additional animals are available for use in the radioimmunoassay (See Appendix 1, Guideline for GFAP radioimmunoassay)[14].

(A) Fixation and Processing of Tissue. The nervous system shall be fixed by in situ perfusion with an appropriate aldehyde fixative. Detailed descriptions of vascular perfusions may be found in Zeman and Innes (1963), Hayat (1970), Spencer and Schaumburg (1980), and Palay and Chan Palay (1974). Any gross abnormalities should be noted. Tissue samples taken shall adequately represent all major regions of the nervous system. Detailed dissection procedures are described in chapter 50 of Spencer and Schaumburg (1980) and in Palay and Chan Palay (1974). The tissue samples should be postfixed and

processed according to standardized published histological protocols such as AFIP (1968), WHO (1986), Spencer and Schaumburg (1980), Bennet et al. (1976), Di Sant Agnese and De Mesy Jensen (1984), or Pender (1985). Tissue blocks and slides shall be appropriately identified when stored. Histological sections shall be stained for hematoxylin and eosin (H&E), or a comparable stain according to standard published protocols such as AFIP(1968), Ralis et al. (1973), or Bennet et al. (1976).

(B) Qualitative Examination[15]. Representative histological sections from the tissue samples shall be examined microscopically by an appropriately trained pathologist for evidence of neuropathological alterations. The nervous system should be thoroughly examined for evidence of any treatment-related neuropathological alterations. Particular attention should be paid to regions known to be sensitive to neurotoxic insult or those regions likely to be affected based on the results of functional tests. Such treatment-related neuropathological alterations should be clearly distinguished from artifacts resulting from influences other than exposure to the test substance. Guidance for both regions to be examined and the types of neuropathological alterations that typically result from toxicant exposure can be found in WHO (1986). A stepwise examination of tissue samples is recommended. In such a stepwise examination, sections from the high dose group are first compared with those of the control group. If no neuropathological alterations are observed in samples from the high dose group, subsequent analysis is not required. If neuropathological alterations are observed in samples from the high dose group, samples from the intermediate and low dose groups are then examined sequentially.

(C) Subjective Diagnosis[16]. If any evidence of neuropathological alterations is found in the qualitative examination, then a subjective diagnosis will be performed for the purpose of evaluating dose-response relationships. All regions of the nervous system exhibiting any evidence of neuropathological changes shall be included in this analysis. Sections from all dose groups from each region will be coded and examined in randomized order without knowledge of the code. The frequency of each type and severity of each lesion will be recorded. After all samples from all dose groups including all regions have been rated, the code will be broken and statistical analysis performed to evaluate dose-response relationships. For each type of dose-related lesion observed, examples of different degrees of severity shall be described. Photomicrographs of typical examples of treatment-related regions are recommended to augment these descriptions. These examples will also serve to illustrate a rating scale, such as 1+, 2+, and 3+ for

the degree of severity ranging from very slight to very extensive.

(e) Data reporting and evaluation. The final test report must include the following information.

(1) Description of equipment and test methods. A description of the general design of the experiment and any equipment used should be provided. This should include a short justification explaining any decisions involving professional judgment.

(i) A detailed description of the procedures used to standardize observations, including the arena and scoring criteria. Procedures for calibrating and assuring the equivalence of activity devices and balancing treatment groups should also be described.

(ii) Positive control data from the laboratory performing the test that demonstrate the sensitivity of the procedures being used. Historical data may be used if all essential aspects of the experimental protocol are the same. Historical control data can be critical in the interpretation of study findings. We encourage submission of such data to facilitate the rapid and complete review of the significance of effects seen.

(2) Results. The following information must be arranged by test group dose level.

(i) In tabular form, data for each animal must be provided showing:

(A) Its identification number;

(B) Its body weight and score on each sign at each observation time, the time and cause of death (if appropriate), total session activity counts, and intra-session subtotals for each day measured.

(ii) Summary data for each group must include:

(A) The number of animals at the start of the test;

(B) The number of animals showing each observation score at each observation time;

(C) The mean and standard deviation for each continuous endpoint at each observation time;

(D) Results of statistical analyses for each measure, where appropriate.

(iii) All neuropathological observations shall be recorded and arranged by test groups. This data may be presented in the following recommended format:

(A) Description of lesions for each animal. For each animal, data must be submitted showing its identification (animal number, sex, treatment, dose, duration), a list of structures examined as well as the location(s), nature, frequency, and severity of lesion(s). Inclusion of photomicrographs is strongly recommended for

demonstrating typical examples of the type and severity of the neuropathological alterations observed is strongly recommended. Any diagnoses derived from neurological signs and lesions including naturally occurring diseases or conditions, shall be recorded.

(B) Counts and incidence of neuropathological alterations by test group. Data shall be tabulated to show:

(1) The number of animals used in each group and the number of animals in which any lesion was found.

(2) The number of animals affected by each different type of lesion, the locations, frequency, and average grade of each type of lesion.

(3) Evaluation of data. The findings from the screening battery should be evaluated in the context of preceding and/or concurrent toxicity studies and any correlated functional and histopathological findings. The evaluation shall include the relationship between the doses of the test substance and the presence or absence, incidence and severity, of any neurotoxic effects. The evaluation should include appropriate statistical analyses, for example, parametric tests for continuous data and non-parametric tests for the remainder. Choice of analyses should consider tests appropriate to the experimental design, including repeated measures. There may be many acceptable ways to analyze data. Statistical analysis comparing total activity counts of treatment vs control animals at each measured time must be made and supplied. The report must include dose-effect curves for observations, motor activity expressed as activity counts, and any gross necropsy findings and lesions observed.

(f) References. For additional background information on this test guideline the following references should be consulted:

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Kinnard, E.J. and Watzman, N. "Techniques utilized in the evaluation of psychotropic drugs on animals activity," Journal of Pharmaceutical Sciences, 55: 995-1012 (1966).

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Reiter, L.W. and MacPhail, R.C. "Motor Activity: A survey of methods with potential use in toxicity testing," Neurobehavorial Toxicology, 1: Suppl. 1, 53-66 (1979).

Robbins, T.W. "A critique of the methods available for the measurement of spontaneous motor activity," Handbook of Psychopharmacology. Vol 7. Eds. Iversen, L.L., Iverson, D.S., Snyder, S.H. New York: Plenum Press. pp. 37-82 (1977).

Spencer, P.S., Schaumburg, H.H. (eds) Experimental and Clinical Neurotoxicology Baltimore: Williams and Wilkins (1980).

Neuro Screen

WHO, Principles and Methods for the Assessment of Neurotoxicity Associated with Exposure to Chemicals (Environmental Health Criteria 60) World Health Organizations Publications Center USA, Albany, New York (1986)

Zeman, W., Innes, J.R. Craigie's Neuroanatomy of the Rat New York: Academic Press. (1963).

NOTES

1. This version of these Neurotoxicity Test Guidelines represents a joint effort of the Offices of Pesticide Programs (OPP) and the Office of Toxic Substances (OTS), in cooperation with many scientists in the Office of Research and Development (ORD), to develop a common set of guidelines for their testing requirements. This OPTS version grew out of the set of guidelines developed and eventually published by OTS (50 FR 39397 9/27/85; amended at 52 FR 19082, 5/20/87). The revisions were initiated by an ad hoc Workgroup of scientists from these 3 offices. They were presented for review by the Scientific Advisory Panel of OPP and made available for public comment for 2 months. Over 30 groups and individuals submitted comments. Many sections of these neurotoxicity guidelines have been revised to take account of these comments. The rationale for some of these general revisions is provided here. What earlier were separate guidelines for the functional observational battery, motor activity, and neuropathology have now been combined into this single guideline both for efficiency and because they were designed to be used together.

2. The Agency recognizes that tests of motor acitivity alone do not provide a complete evaluation of the effects of a chemical on the nervous system. However, the automated test of motor activity will provide an objective assessment of neurobehavioral function, as well as the only measurement of habituation, which is an indication of the organism's ability to adapt to its environment.

3. The power calculations to determine group size for motor activity have been deleted. Group sizes of ten/sex will be sufficient for well designed and executed studies. Poorly designed and/or executed studies may be judged invalid. The original intent was to allow for flexibility in the use of devices with different operating characteristics (larger variance in measures necessitate larger group sizes). Comments were mostly negative, based on concerns about perceived uncertainty of the adequacy of a sample size until a study was complete.

4. Positive control data need only be generated approximately once every year as long as most conditions remain the same. Each observer should conduct such studies as part of their training. The untreated control data from training studies could then be submitted as part of the laboratory's historical control data.

5. The adequacy of the high dose for hazard identification is one of the most critical issues of experimental design. The high dose criteria in these guidelines now include both a limit dose and " significant neurotoxic effects or other clearly toxic effects".

These revised criteria are very similar to those for systemic studies and are intended by this to facilitate combined studies.

We now also include some guidance for dose selection in acute studies based on a benchmark dose criterion and fractions thereof.
This is intended to provide an example of one operational means of establishing a set of acute doses and to reduce the number of animals used for estimates of lethal doses.

These 3 dose studies are intended to both identify effects of exposure and to estimate dose levels without adverse effect. We have added language to encourage greater emphasis on obtaining dose response data, e.g., equally spaced doses and lesser emphasis on a low dose totally without effect. This was done for 2 reasons. First, the presence or absence of dose-related changes can be critical in the evaluation of effects of exposure. Second, various methods using an ED10 as the basis for estimating reference doses are increasingly discussed by many authors.

We strongly urge sponsors to seek guidance from the Agency before initiating their studies and to provide a rationale for dose selection in these studies.

Several commenters were concerned about the interpretation of effects seen at levels where other significant toxicity was present and questioned the need for or efficiency of testing at such doses. First, in OPP, the 90 day Neurotoxicity Study is intended as a screen prior to inclusion of neurotoxicity tests in chronic studies. In OTS, subchronic non-oncogenicity studies are generally considered sufficient for evaluating chronic toxicity. Thus the doses of a 90 day neurotoxicity study for either office should be maximized to encourage the identification of chronic effects.

Further, concurrent toxicity does not obviate the need to identify other kinds of effects that may be more important in different situations, either for other groups or after different exposure regimens. Ultimately, all of the targets of a toxicant may be important for identifying affected individuals or be the critical effect under a variety of exposure conditions or in different groups.

6. The term benchmark dose as defined here is for the purpose of dose selection and should not be confused with the use of this term for the purpose of establishing a common dose such as an ED10 for extrapolation in risk assessment.

7. The time of peak effect refers to the time within approximately 8 hours after dosing and was intended to help maximize detection of effects of acute exposure.

8. For repeated exposure studies using routes other than through the diet, the intent of testing before the daily dose is to minimize the impact of that day's dose. We recognize that for some materials, residual material from preceding dose or doses may be the source of observed effects, but these are part of the effects of concern. In some studies, examination of animals following exposure may help to further describe the duration of such effects.

9. Not all rats must be tested in one day, but time of testing should be balanced across groups, and for any other potential confounds, e.g., sex.

10. Measures of reactivity refer to the subject's reaction to some external stimulus, e.g., removal from the cage or handling, while arousal or state of alertness refers to the behavior of the undisturbed subject observed in the open field. This is often described more technically as the distinction between respondent and operant behaviors.

11. Landing foot splay and grip strength do not measure the same function. These tests are viewed as complementary, and having both will aid in the interpretation of data.

12. The goal of the procedures outlined for the preparation and processing of tissue samples is to optimally preserve tissue morphology for microscopic examination. The higher resolution obtainable in plastic embedded tissue is considered to optimize the detection of a number of types of lesions, particularly in the peripheral nervous system. In contrast, paraffin-embedded material is more amenable to sampling large regions of the nervous system and is considered optimal for a variety of special stains that may be useful in characterizing neuropathological alterations. Several organizations felt the requirement for separate animals for plastic and paraffin-embedding of tissue samples was excessive. Furthermore, commentors presented views that differences in plastic and paraffin techniques did not

require the use of separate animals and that in an appropriate tiered evaluation scheme both plastic and paraffin-embedding could be used with tissue samples from the same animals. The requirement for separate animals is now eliminated, with paraffin being acceptable and plastic being recommended.

13. The higher resolution obtainable in plastic embedded tissue is considered to optimize the detection of a number of types of lesions, particularly in the peripheral nervous system. Paraffin embedded material is more amenable to sampling large regions of the nervous system and is considered optimal for a variety of special stains that may be useful in characterizing neuropathological alterations.

14. Although, EPA believes that the GFAP assay has been shown to be sensitive to the neurotoxic effects of agents in both the adult and developing nervous systems, this assay has been deleted at this time. However, the Agency will continue using this assay experimentally and encourages others to do so, as well, in an effort to obtain additional validation of its use as a means to assess the neurotoxic potential of agents. In addition, if GFAP immunohistochemistry is used as a special stain in the neuropathology segment of the testing protocol and evidence of a glial response to toxicant injury is observed, application of the radioimmunoassay is encouraged in order to provide objective, quantitative dose-response data.

15. The Agency received some comments that the list of specified regions of the nervous system to be examined was inadequate, while others felt it was too detailed. Moreover, comments were received that argued that the list of potential types of neuropathological alterations also was too restricted. These lists were intended to serve as guidance. Since they appear to be subject to misinterpretation, the requirement for a thorough examination of the nervous system for any evidence of neuropathological alteration is now explicitly stated. In addition, a list of all structures examined is required in the final report. The requirement for examination of more than one section per region, however, has now been deleted.

16. The purpose of the semi-quantitative analysis is to evaluate the relationship between the incidence and severity of the neuropathological alterations and the exposure. Since the rating scale is by necessity subjective, it is necessary to ensure that any bias resulting from the previous qualitative examination of the tissue samples is minimized. Several organizations commented

that blind evaluation of tissue sections should not be required. In the initial qualitative analysis, in which the types of lesions and regions affected are first identified, blind evaluation is not required. However, in the semi-quantitative analysis in which the dose-response relationship is evaluated, it is imperative that the evaluation be as objective as possible. Moreover, since the semi-quantitative analysis focuses on a limited number of regions for lesions previously described in the qualitative analysis, blind reading is required to ensure objectivity. Thus, it is required that the subjective rating of the severity and incidence be performed without knowledge of treatment.

17. The data and analyses supplied in the report must be evaluated by Agency risk assessors. Thus, the report must be sufficiently detailed for the Agency to evaluate the quality of the study. Since no list of regions to be examined is outlined in the guideline, a list of regions examined must be supplied with the report. Similarly, an adequate description of lesions observed must be supplied also. The Agency received comments that the requirement of photomicrographs to document neuropathological alterations was extremely costly. The Agency has decided to recommend, rather than require, the use of photomicrographs to aid in the description of typical examples of treatment-related lesions.

APPENDIX 1

GUIDELINE FOR ASSAYING GLIAL FIBRILLARY ACIDIC PROTEIN

(a) Purpose. Chemical-induced injury of the nervous system is associated with astrocytic hypertrophy at the site of damage (see O'Callaghan, 1988). Assays of glial fibrillary acid protein (GFAP), the major intermediate filament protein of astrocytes, can be used to document this response. To date, a diverse variety of chemical insults known to be injurious to the central nervous system have been shown to increase GFAP. Moreover, increases in GFAP can be seen at dosages below those necessary to produce cytopathology as determined by routine Nissl stains (standard neuropathology). Thus, it appears that assays of GFAP represent a sensitive approach for documenting the existence and location of chemical-induced injury of the central nervous system.

(b) Principle of the test method. This guideline will describe the conduct of a radioimmunoassay for measurement of the amount of GFAP in the brain of exposed and control animals. It is based on modifications (O'Callaghan & Miller 1985, O'Callaghan 1987, O'Callaghan and Miller, 1988) of the dot-immunobinding procedure described by Jahn et al. (1984). Briefly, samples are assayed for total protein, diluted in dot-immunobinding buffer, and applied to nitrocellulose sheets. The spotted sheets are then fixed, blocked, washed, and incubated in anti-GFAP and [125I] Protein A. Bound protein A is then quantified by gamma spectrometry. In lieu of purified protein standards, standard curves are constructed from dilution of a single control sample. By comparing the immunoreactivity of individual samples (both control and treated groups) with that of the sample used to generate the standard curve, the relative immunoreactivity of each sample is obtained. The immunoreactivity of the control groups is normalized to 100% and all data are expressed as a percentage of control. This biochemical test is intended to be used in conjunction with behavioral and pathological studies as part of the screening battery that includes the functional observational battery, motor activity and histopathology.

(c) Test procedure. (1) Animal selection. (i) Species and strain. Test shall be performed in the species being used in other tests for neurotoxicity. This will generally be the laboratory rat.

(ii) Age. Based on the other concurrent testing young adult rats shall be used.

(iii) Number of animals. At least 6 animals per dose shall be used.

(2) Materials: [125I] Protein A (2-10 uCi/ug), Antisera to GFAP, Nitrocellulose paper (0.1 or 0.2 um pore size), a sample

application template (optional, e.g. Minifold II, Schleicher & Schuell, Keene NH), plastic incubation trays.

(3) Study conduct. (i) Tissue Preparation. Animals are killed by decapitation 72 hours after the last dose. The brain is excised from the skull. The following six regions are then dissected freehand: cerebellum, cerebral cortex, hippocampus, striatum, thalamus/hypothalamus, and the rest of the brain. Each region is then weighed and homogenized in 10 volumes of hot (70-90 degrees C) 1% (w/v) sodium dodecyl sulfate (SDS). Homogenization is best achieved through sonic disruption. A motor driven pestle inserted into a tissue grinding vessel is a suitable alternative. The homogenized samples can then be stored frozen at -70 C for at least 4 years without loss of GFAP content.

(ii) Total Protein Assay. Aliquots of the tissue samples are assayed for total protein using the method of Smith et. al. (1985). This assay is available in kit form (Pierce Chemical Company, Rockford, IL).

(iii) Sample Preparation. Dilute tissue samples in sample buffer (120 mM KCl, 20 mM NaCl, 2 mM NaHCO3), 2 mM MgCl2), 5 mM Hepes, pH 7.4, 0.7% Triton X-100) to a final concentration of 0.25 mg total protein per ml (5 ug/20 ul).

(iv) Preparation of Standard Curve. Dilute a single control sample in sample buffer to give at least five standards, between 1 and 10 ug total protein per 20 ul. The suggested values of total protein per 20 ul sample buffer are: 1.25, 2.50, 3.25, 5.0, 6.25, 7.5, 8.75, and 10.0 ug.

(v) Preparation of Nitrocellulose Sheets. Nitrocellulose sheets of 0.1 or 0.2 micron pore size are rinsed by immersion in distilled water for 5 minutes and then air dried.

(vi) Sample Application. Samples can be spotted onto the nitrocellulose sheets free-hand or with the aid of a template. For free-hand application, draw a grid of squares approximately 2 cm by 2 cm on the nitrocellulose sheets using a soft pencil. Spot 5-10 ul portions to the center of each square for a total sample volume of 20 ul. For template aided sample application a washerless microliter capacity sample application manifold is used. Position the nitrocellulose sheet in the sample application device as recommended by the manufacturer and spot a 20 ul sample in one application. Do not wet the nitrocellulose or any support elements prior to sample application. Do not apply vacuum during or after sample application. After spotting samples (using either method), let the sheets air dry. The sheets can be stored at room temperature for several days after sample application.

(vii) Standard Incubation Conditions. These conditions have been described by Jahn et al. (1984). All steps are carried out at room temperature on a flat shaking platform (one complete excursion every 2-3 seconds). For best results do

not use rocking or orbital shakers. Perform the following steps in enough solution to cover the nitrocellulose sheets to a depth of 1 cm.

(A) Incubate 20 minutes in fixer (25% v/v isopropanol, 10% v/v acetic acid).

(B) Discard fixer, wash several times in deionized water to eliminate the fixer, and then incubate for 5 minutes in Tris-buffered saline (TBS, 200 mM NaCl, 60 mM Tris-HCl pH 7.4).

(C) Discard TBS and incubate 1 hour in blocking solution (0.5% gelatin (w/v) in TBS.

(D) Discard blocking solution and incubate for 2 hours in antibody solution (anti-GFAP antiserum diluted to the desired dilution in blocking solution containing 0.1% Triton X-100). Serum antibovine GFAP, which cross reacts with GFAP from rodents and humans, can be obtained commercially (e.g. Dako Corp.) and used at a dilution of 1:500.

(E) Discard antibody solution, wash in 4 changes of TBS for 5 minutes each time. Then wash in TBS for 10 minutes.

(F) Discard TBS and incubate in blocking solution for 30 minutes.

(G) Discard blocking solution and incubate for 1 hour in Protein A solution ([125I]-labeled Protein A diluted in blocking solution containing 0.1% Triton X-100, sufficient to produce 2000 cpm per 10 ul of protein A solution).

(H) Remove protein A solution (it can be reused once). Wash in 0.1% Triton X-100 in TBS (TBSTX) for 5 minutes, 4 times. Then wash in TBSTX for 2-3 hours for 4 additional times. An overnight wash in a larger volume can be used to replace the last 4 washes.

(I) Hang sheets up to dry, cut out squares or spots and count radioactivity in a gamma counter.

(viii) Expression of data. Compare radioactivity counts for samples obtained from control and treated animals with counts obtained from the standard curve. By comparing the immunoreactivity (counts) of each sample with that of the standard curve, the relative amount of GFAP in each sample can be determined and expressed as a percent of control.

(d) Data Reporting and Evaluation. (1) Test Report. The final test report shall include the following information:

(i) Body weight and brain region weights at time of sacrifice for each subject tested.

(ii) Indication of whether each subject survived to sacrifice or time of death.

(iii) Data from control animals and blank samples.

(iv) Statistical evaluation of results.

(2) Evaluation of Results. (i) Results shall be evaluated in terms of the extent of change in the amount of GFAP

as a function of treatment and dose. GFAP assays (of any brain region) from 6 samples typically result in a standard error of the mean of +/- 5%. Chemical-induced increase of GFAP of 115% of control are likely to be statistically significant.

(ii) Results of this assay shall be compared to and evaluated with behavioral and histopathological data.

(e) References. For additional background information on this test guideline the following references should be consulted:

Brock, T.O. and O'Callaghan, J.P. 1987. Quantitative changes in the synaptic vesicle proteins, synapsin I and p38 and the astrocyte specific protein, glial fibrillary acidic protein, are associated with chemical-induced injury to the rat central nervous system. J. Neurosci. 7:931-942

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O'Callaghan, J. P. and Miller, D. B. 1988. Acute exposure of the neonatal rat to triethyltin results in persistent changes in neurotypic and gliotypic proteins. J. Pharmacol. Exp. Ther. 244:368-378.

O'Callaghan, J. P. and Miller, D. B. 1985. Cerebellar hypoplasia in the Gunn rat is associated with quantitative changes in neurotypic and gliotypic proteins. J. Pharmacol. Exp. Ther. 234:522-532.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A. K. Gartner, F. H., Provenzano, M.D., Fujimoto, E. K. , Goeke, N.M. Olson, B.J., Klenk, D.C. 1985. Measurement of protein using bicinchoninic acid. Annal. Biochem. 150:76-85

DEVELOPMENTAL NEUROTOXICITY STUDY[1]

(a) Purpose. In the assessment and evaluation of the toxic characteristics of a chemical substance or mixture ("test substance"), determination of the potential for developmental neurotoxicity is important. This study is designed to develop data on the potential functional and morphological hazards to the nervous system which may arise in the offspring from exposure of the mother during pregnancy and lactation.

(b) Principle of the test method. The test substance is administered to several groups of pregnant animals during gestation and early lactation, one dose level being used per group. Offspring are randomly selected from within litters for neurotoxicity evaluation. The evaluation includes observations to detect gross neurologic and behavioral abnormalities, determination of motor activity, response to auditory startle, assessment of learning, neuropathological evaluation, and brain weights. This protocol may be used as a separate study, as a follow-up to a standard developmental toxicity and/or adult neurotoxicity study, or as part of a two-generation reproduction study, with assessment of the offspring conducted on the F_2 generation.

(c) Test procedure. (1) Animal selection. (i) Species and strain. Testing should be performed in the rat. Because of its differences in timing of developmental events compared to strains that are more commonly tested in other developmental and reproductive toxicity studies, it is preferred that the Fischer 344 strain not be used. If a sponsor wishes to use the Fischer 344 rat or a mammalian species other than the rat, ample justification/reasoning for this selection must be provided.

(ii) Age. Young adult (nulliparous females) animals shall be used.

(iii) Sex. Pregnant female animals shall be used at each dose level.

(iv) Number of animals. (A) The objective is for a sufficient number of pregnant rats to be exposed to the test substance to ensure that an adequate number of offspring are produced for neurotoxicity evaluation. At least 20 litters are recommended at each dose level. For behavioral tests, one female and one male pup per litter shall be randomly selected and assigned to one of the tests.

(B) On postnatal day 4, the size of each litter should be adjusted by eliminating extra pups by random selection to yield, as nearly as possible, 4 male and 4 females per litter. Whenever the number of pups of either sex prevents having four of each sex per litter, partial adjustment (for example, 5 males and 3 females) is permitted. Testing is not appropriate for litters of less than 7 pups. Elimination of runts only is not appropriate. Individual pups should be

identified uniquely after standardization of litters. A method that may be used for identification can be found in Adams et al. (1985).

(C) Assignment of animals for behavioral tests, brain weights, and neuropathological evaluations. After standardization of litters, one male and one female from each litter shall be randomly assigned to one of the following tests: motor activity; auditory startle; and learning and memory, in weanling and adult animals. On postnatal day 11, either one male or one female pup from each litter (total of 10 males and 10 females/dose group) shall be sacrificed. Brain weights shall be measured in all of these pups and, of these pups, 6/sex/dose shall be selected for neuropathological evaluation. At the termination of the study, either one male or one female from each litter (total of 10 males and 10 females/dose group) shall be sacrificed and brain weights shall be measured. An additional group of 6 animals/sex/dose group (one male or one female per litter) shall be sacrificed at the termination of the study for neuropathological evaluation.

(2) Control groups. A concurrent control group(s) is (are) required. This group shall be a sham-treated group or, if a vehicle is used in administering the test substance, a vehicle control group. The vehicle shall neither be developmentally toxic nor have effects on reproduction. Animals in the control group(s) shall be handled in an identical manner to test group animals.

(3) Dose levels and dose selection. (i) At least 3 dose levels of the test substance plus a control group (vehicle control, if a vehicle is used) shall be used.

(ii) If the test substance has been shown to be developmentally toxic either in a standard developmental toxicity study or in a pilot study, the highest dose level shall be the maximum dose which will not induce in utero or neonatal death or malformations sufficient to preclude a meaningful evaluation of neurotoxicity[2].

(iii) If a standard developmental toxicity study has not been conducted, the highest dose level, unless limited by the physico-chemical nature or biological properties of the substance, shall induce some overt maternal toxicity, but shall not result in a reduction in weight gain exceeding 20% during gestation and lactation.

(iv) The lowest dose should not produce any grossly observable evidence of either maternal or developmental neurotoxicity.

(v) The intermediate dose(s) shall be equally spaced between the highest and lowest doses used.

(4) Dosing period[3]. Day 0 of gestation is the day on which a vaginal plug and/or sperm are observed. The dosing period shall cover the period from day 6 of gestation through day 10 postnatally. Dosing should not occur on the day of

parturition in those animals who have not completely delivered their offspring.

(5) Administration of the test substance. The test substance or vehicle shall be administered orally. Other routes of administration may be acceptable, on a case-by-case basis, with ample justification/reasoning for this selection. The test substance or vehicle shall be administered at the same time each day. The animals shall be weighed periodically and the dosage to be administered based on the most recent weight determination.

(6) Observation of dams. (i) A gross examination of the dams shall be made at least once each day before daily treatment. The animals shall be observed by trained technicians, who are unaware of the animal's treatment, using standardized procedures to maximize inter-observer reliability. Where possible, it is advisable that the same observer be used to evaluate the animals in a given study. If this is not possible, some demonstration of inter-observer reliability is required.

(ii) During the treatment and observation periods, observations shall include:

(A) Assessment of signs of autonomic function, including but not limited to:

(1) ranking of the degree of lacrimation and salivation, with a range of severity scores from none to severe;

(2) presence or absence of piloerection and exophthalmus;

(3) ranking or count of urination and defecation, including polyuria and diarrhea;

(4) pupillary function such as constriction of the pupil in response to light or a measure of pupil size;

(5) degree of palpebral closure, e.g., ptosis.

(B) Description, incidence, and severity of any convulsions, tremors, or abnormal movements.

(C) Description and incidence of posture and gait abnormalities.

(D) Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

(iii) Signs of toxicity shall be recorded as they are observed, including the time of onset, degree, and duration.

(iv) Animals shall be weighed at least weekly and on the day of delivery and postnatal days 11 and 21 (weaning); such weights shall be recorded.

(v) The day of delivery of litters shall be

recorded and considered as postnatal day 0.

(7) Study conduct. (i) Observation of offspring.

(A) All offspring shall be examined cage-side at least daily for gross signs of mortality or morbidity.

(B) All offspring shall be examined outside the cage for gross signs of toxicity whenever they are weighed or removed from their cages for behavioral testing. The offspring shall be observed by trained technicians, who are unaware of the animals' treatment, using standardized procedures to maximize inter-observer reliability. Where possible, it is advisable that the same observer be used to evaluate the animals in a given study. If this is not possible, some demonstration of inter-observer reliability is required. At a minimum, the end points outlined in paragraph (6) (ii) shall be monitored as appropriate for the developmental stage being observed.

(C) Any gross signs of toxicity in the offspring shall be recorded as they are observed, including the time of onset, degree, and duration.

(ii) Developmental landmarks. Live pups shall be counted and each pup within a litter shall be weighed individually at birth or soon thereafter, and on postnatal days 4, 11, 17, 21 and at least once every two weeks thereafter. The age of vaginal opening and preputial separation shall be determined. General procedures for these determinations may be found in Adams et al. (1985), and Korenbrot et al. (1977),respectively.

(iii) Motor activity. Motor activity shall be monitored specifically on postnatal days 13, 17, 21, and 60 ($\pm$2 days)[5]. Motor activity must be monitored by an automated activity recording apparatus. The device must be capable of detecting both increases and decreases in activity, (i.e., baseline activity as measured by the device must not be so low as to preclude detection of decreases nor so high as to preclude detection of increases in activity). Each device shall be tested by standard procedures to ensure, to the extent possible, reliability of operation across devices and across days for any one device. In addition, treatment groups must be balanced across devices. Each animal shall be tested individually. The test session shall be long enough for motor activity to approach asymptotic levels by the last 20 percent of the session for non-treated control animals. All sessions shall have the same duration. Treatment groups shall be counter-balanced across test times. Animals' activity counts shall be collected in equal time periods of no greater than 10 minutes duration. Efforts shall be made to ensure that variations in the test conditions are minimal and are not systematically related to treatment. Among the variables that can affect motor activity are sound level, size and shape of the test cage, temperature, relative humidity, light conditions, odors, use of home cage or novel test cage, and environmental distractions. Additional information on

the conduct of a motor activity study may be obtained in the Office of Pesticides and Toxic Substances Neurotoxicity Screening Battery Guideline.

(iv) Auditory startle test. An auditory startle habituation test shall be performed on the offspring on days 22 and 60 ± 2. Details on the conduct of this testing may be obtained in Adams et al. (1985). In performing the auditory startle task, the mean response amplitude on each block of 10 trials (5 blocks of 10 trials per session on each day of testing) shall be made. While use of pre-pulse inhibition is not a requirement, it is highly recommended. Details on the conduct of this test may be obtained from Ison (1984).

(v) Learning and memory tests. A test of associative learning and memory shall be conducted around the time of weaning (postnatal day 21-24) and at adulthood (postnatal day 60 ± 2). The same or separate test(s) may be used at these two stages of development. Some flexibility is allowed in the choice of test(s) for learning and memory in weanling and adult rats. However, the test(s) must be designed so as to fulfill two criteria. First, learning must be assessed either as a change across several repeated learning trials or sessions, or, in tests involving a single trial, with reference to a condition that controls for non-associative effects of the training experience. Second, the test(s) shall include some measure of memory (short-term or long-term) in addition to original learning (acquisition), but note that this measure of memory cannot be reported in the absence of a measure of acquisition obtained from the same test. If the test(s) of learning and memory reveal(s) an effect of the test compound, it may be in the best interest of the sponsor to conduct additional tests to rule out alternative interpretations based on alterations in sensory, motivational, and/or motor capacities. In addition to the above two criteria, it is recommended that the test of learning and memory be chosen on the basis of its demonstrated sensitivity to the class of compound under investigation, if such information is available in the literature. In the absence of such information, examples of tests that could be made to meet the above criteria include: delayed-matching-to-position, as described for the adult rat in Bushnell (1988) and for the infant rat in Green and Stanton (1988, Experiment 2); olfactory conditioning, as described in Kucharski and Spear (1984, Experiment 3); and acquisition and retention of schedule-controlled behavior, e.g., Cory-Slechta et al., 1983, and Campbell and Haroutunian, 1981. Additional tests for weanling rats are described in Spear and Campbell (1978) and Krasnegor et al. (1986), and for adult rats in Miller and Eckerman (1986).

(iv) Neuropathology[6]. Neuropathological evaluation shall be conducted on animals on postnatal day 11 and at the termination of the study. At 11 days of age, one male or female pup shall be removed from each litter such that equal

numbers of male and female offspring are removed from all litters combined. Of these, 6 male and 6 female pups will be sacrificed for neuropathological analysis. The pups will be killed by exposure to carbon dioxide and immediately thereafter the brains shall be removed, weighed, and immersion fixed in an appropriate aldehyde fixative. The remaining animals will be sacrificed in a similar manner and immediately thereafter their brains removed and weighed. At the termination of the study, one male or one female from each litter will be killed by exposure to carbon dioxide and immediately thereafter the brain shall be removed and weighed. In addition, 6 animals/sex/dose group (one male or female per litter) shall be sacrificed at the termination of the study for neuropathological evaluation. Neuropathological analysis of animals sacrificed at the termination of the study shall be performed in accordance with the Office of Pesticides and Toxic Substances Neurotoxicity Screening Battery. Neuropathological evaluation of animals sacrificed on postnatal day 11 and at termination of the study shall include a qualitative analysis and semi-quantitative analysis as well as simple morphometrics.

(A) Fixation and Processing of Tissue Samples for Postnatal Day 11 Animals. Immediately following removal, the brain shall be weighed and immersion fixed in an appropriate aldehyde fixative. The brains should be postfixed and processed according to standardized published histological protocols a such as the AFIP (1968), Spencer and Schaumburg (1980), Di Sant Agnese and De Mesy Jensen (1984), or Pender (1985). Paraffin embedding is acceptable but plastic embedding is preferred and recommended. Tissue blocks and slides shall be appropriately identified when stored. Histological sections shall be stained for hematoxylin and eosin, or a similar stain according to standard published protocols such as AFIP (1968), Ralis et al. (1973), or Bennet et al. (1976). For animals sacrificed at the termination of the study, methods for fixation and processing of tissue samples are provided in the section "Fixation and Processing of Tissue Samples" in the OPTS Neurotoxicity Screening Battery.

(B) Qualitative Analysis. The purposes of the qualitative examination are: one, to identify regions within the nervous system exhibiting evidence of neuropathological alterations; two, to identify types of neuropathological alterations resulting from exposure to the test substance; and three, to determine the range of severity of the neuropathological alterations. Representative histological sections from the tissue samples shall be examined microscopically by an appropriately trained pathologist for evidence of neuropathological alterations. The following stepwise procedure is recommended for the qualitative analysis. First, sections from the high dose group are compared with those of the control group. If no evidence of neuropathological

alterations are found in animals of the high dose group, no further analysis is required. If evidence of neuropathological alterations are found in the high dose group, then animals from the intermediate and low dose group are examined. Subject to professional judgement and the kind of neuropathological alterations observed, it is recommended that additional methods such as Bodian's or Bielchowsky's silver methods and/or immunohistochemistry for glial fibrillary acid protein be used in conjunction with more standard stains to determine the lowest dose level at which neuropathological alterations are observed[7]. Evaluation of postnatal day 11 pups is described in sections (1) and (2) below. For animals sacrificed at the termination of the study, the regions to be examined and the types of alterations that shall be assessed are identified in the section "Qualitative Examination" in the OPTS Neurotoxicity Screening Battery.

(1) Regions to be Examined. The brains should be examined for any evidence of treatment-related neuropathological alterations and adequate samples should be taken from all major brain regions [e.g., olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain (tectum, tegmentum, and cerebral peduncles), brainstem and cerebellum] to insure a thorough examination.

(2) Types of Alterations. Guidance for neuropathological examination for indications of developmental insult to the brain can be found in Friede (1975) and Suzuki (1980). In addition to more typical kinds of cellular alterations (e.g., neuronal vacuolation, degeneration, necrosis) and tissue changes (e.g., astrocytic proliferation, leukocytic infiltration, and cystic formation) particular emphasis should be paid to structural changes indicative of developmental insult including but not restricted to:

a) gross changes in the size or shape of brain regions such as alterations in the size of the cerebral hemispheres or the normal pattern of foliation of the cerebellum;

b) the death of neuronal precursors, abnormal proliferation, or abnormal migration, as indicated by pyknotic cells or ectopic neurons, or gross alterations in regions with active proliferative and migratory zones, alterations in transient developmental structures [e.g., the external germinal zone of the cerebellum, see Miale and Sidman (1961) for discussion];

c) abnormal differentiation, while more apparent with special stains, may also be indicated by shrunken and malformed cell bodies;

d) evidence of hydrocephalus, in particular enlargement of the ventricles, stenosis of the cerebral aqueduct and general thinning of the cerebral hemispheres.

(C) Subjective Diagnosis. If any evidence of neuropathological alterations is found in the qualitative examination, then a subjective diagnosis will be performed for the purpose of evaluating dose-response relationships. All regions of the brain exhibiting any evidence of neuropathological changes shall be included in this analysis. Sections of each region from all dose groups will be coded as to treatment and examined in randomized order. The frequency of each type and the severity of each lesion will be recorded. After all sections from all dose groups including all regions have been rated, the code will be broken and statistical analyses performed to evaluate dose-response relationships. For each type of dose-related lesion observed, examples of different ranges of severity shall be described. The examples will serve to illustrate a rating scale, such as 1+, 2+, and 3+ for the degree of severity ranging from very slight to very extensive.

(D) Simple Morphometric Analysis. Since the disruption of developmental processes is sometimes more clearly reflected in the rate or extent of growth of particular brain regions, some form of morphometric analysis shall be performed on postnatal day 11 and at the termination of the study to assess the structural development of the brain. At a minimum, this would consist of a reliable estimate of the thickness of major layers at representative locations within the neocortex, hippocampus and cerebellum. For guidance on such measurements see Rodier and Gramann (1971).

(e) Data collection, reporting, and evaluation. The following specific information shall be reported:

(1) Description of test system and test methods. A description of the general design of the experiment should be provided. This shall include:

(i) A detailed description of the procedures used to standardize observations and procedures as well as operational definitions for scoring observations.

(ii) Positive control data from the laboratory performing the test that demonstrate the sensitivity of the procedures being used. These data do not have to be from studies using prenatal exposures. However, the laboratory must demonstrate competence in evaluating effects in neonatal animals perinatally exposed to chemicals and establish test norms for the appropriate age group.

(iii) Procedures for calibrating and ensuring the equivalence of devices and the balancing of treatment groups in testing procedures.

(iv) A short justification explaining any decisions involving professional judgment.

(2) Results. The following information must be arranged by each treatment and control group:

(i) In tabular form, data for each animal must be

provided showing:

(A) Its identification number and the litter from which it came.

(B) Its body weight and score on each developmental landmark at each observation time; total session activity counts and intrasession subtotals on each day measured; auditory startle response amplitude per session and intrasession amplitudes on each day measured; appropriate data for each repeated trial (or session) showing acquisition and retention scores on the test(s) of learning and memory on each day measured; time and cause of death (if appropriate), any neurological signs observed, a list of structures examined as well as the location(s), nature, frequency, and extent of lesion(s); and brain weights. Inclusion of photomicrographs demonstrating typical examples of the type and extent of the neuropathological alterations observed is recommended. Any diagnoses derived from neurological signs and lesions, including naturally-occurring diseases or conditions, should also be recorded.

(ii) Summary data for each treatment and control group must include:

(A) The number of animals at the start of the test.

(B) The body weights of the dams during gestation and lactation.

(C) Litter size and mean weight at birth.

(D) The number of animals showing each abnormal sign at each observation time.

(E) The percentage of animals showing each abnormal sign at each observation time.

(F) The mean and standard deviation for each continuous end point at each observation time. These will include body weight, motor activity counts, auditory startle responses, performance in learning and memory test(s), regional brain weights and whole brain weights (both absolute and relative).

(G) The number of animals in which any lesion was found.

(H) The number of animals affected by each different type of lesion, the location, frequency and average grade of each type of lesion for each animal.

(I) The values of all morphometric measurements made for each animal listed by treatment group.

(3) Evaluation of data. An evaluation of test results must be made. The evaluation shall include the relationship between the doses of the test substance and the presence or absence, incidence, and extent of any neurotoxic effect. The evaluation shall include appropriate statistical analyses. The choice of analyses shall consider tests appropriate to the experimental design and needed adjustments for multiple

comparisons. The evaluation shall include the relationship, if any, between observed neuropathological and behavioral alterations.

(e) References. For additional background information on this test guideline the following references should be consulted:

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Devel Neuro

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Rodier, P. M., Gramann, W. J. Morphologic effects of interference with cell proliferation in the early fetal period. Neurobehavioral Toxicology 1:128-135 (1971).

Spear, N. E., Campbell, B. A. (eds.) Ontogeny of Learning and Memory. New Jersey: Erlbaum (1979).

Spencer, P. S., Schaumburg, H. H. (eds.) Experimental and Clinical Neurotoxicology. Baltimore: Williams and Wilkins (1980)

Suzuki, K. Special vulnerabilities of the developing nervous system. In: Experimental and Clinical Neurotoxicology, P. S. Spencer and H. H. Schaumburg (eds.) Baltimore: Williams and Wilkins, pp. 48-61 (1980).

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NOTES

Response to Public and SAP Comment
on the
Developmental Neurotoxicity Study

EPA's Work Group on Developmental Neurotoxicology would like to acknowledge and thank the individuals and organizations that provided comment on the "Developmental Neurotoxicity Study" protocol. The Work Group has reviewed all of the comments and taken them into consideration in revising the protocol. Responding to every individual comment is beyond the scope of this effort. Therefore, response will be limited to those comments that were raised by more than one individual or organization and which significantly impact the design of the study.

1. COMMENT: The developmental neurotoxicity protocol is too complex and should be restricted to those agents for which there is sufficient justification to undergo such testing. A simpler, "tier 1" test should be developed that could be used more routinely.

EPA RESPONSE: The Agency had a number of discussions on this issue. At first, the Agency considered development of a two-tier approach within the confines of a single study design. That is, measurements would have been carried out periodically during postnatal development. The "tier 1" component would have been the study carried until postnatal day 24 and the "tier 2" component would have been an extension of "tier 1" into adulthood. Whether or not the "tier 2" component would have been conducted would have depended on the analysis of the data up to postnatal day 24. This proposal was presented at several scientific meetings. However, it was criticized by the public for several reasons. First, concerns were raised that assessments only through the time of weaning may not be sufficiently sensitive to detect all potential developmental neurotoxicants. That is, unless assessments were carried out into adulthood, there would be a possibility that some potential developmental neurotoxicants would not be identified. Second, there was a strong sentiment among scientists from industry and contract laboratories that the assessments from tier 1 would not be completed in time for a decision to be made as to whether or not to proceed with the tier 2. In light of these concerns, the Agency has decided to publish the protocol as a single test to be conducted in its entirety, as it had been proposed. In the meantime, the Agency will be considering more feasible approaches

for development as a "screen" or "tier 1" type of protocol and encourages the development of screening methodologies by scientists working in this area.

2.COMMENT: Reference to the highest dose level as inducing "some overt maternal toxicity" was considered to be excessive. Some suggested that the highest dose level should be below the "threshold" for "minimal maternal toxicity."

EPA RESPONSE: The Agency disagrees. The protocol further qualifies that the highest dose "shall not result in a reduction in weight gain exceeding 20% during gestation and lactation" and that it "will not induce in utero or neonatal death or malformations sufficient to preclude a meaningful evaluation of neurotoxicity." This represents as minimal a toxic level as one could require in order to ensure that the agent has been adequately tested across an appropriate range of dose levels. The Agency is satisfied with this requirement in the guideline and does not believe a change is necessary.

3.COMMENT: The specified duration of dosing, that is day 6 of gestation through day 21 postnatally, is excessive. Dosing of the dams postnatally should not be required because of potential effects on maternal behavior, milk production, or milk let-down, or sequestration of the agent in the milk with transfer to the pup, any of which may alter maternal-neonatal interactions. Furthermore, it was noted that pups would be undergoing observations and testing while potentially being exposed to the agent via the milk; thus, alterations in these measurements may be due to pharmacologic action of the agent rather than to a neurotoxic effect. It would not be possible to distinguish these effects.

EPA RESPONSE: After careful consideration of all of the issues, the Agency has revised the protocol so that the period of exposure is now from day 6 of gestation through day 10 postnatally. The rationale behind this revision was as follows:

First, the Agency strongly believes that dosing should continue into the postnatal period for several reasons. These include: 1) several major events that occur prenatally in the nervous system of the human are still going through critical stages postnatally in the rat, and 2) exposure to the still developing organism may occur when agents are transferred from the mother to the offspring via the milk. An alternaltive postnatal exposure route that has been suggested is direct dosing of neonates, but the Agency believes that more work is needed to develop better methods before this should be adopted.

Second, although the Agency feels strongly that dosing

should continue into the postnatal period for the reasons identified above, it recognizes the potential confounding factors with postnatal dosing that were raised in the public comments and has, therefore, adopted a compromise position. The Agency has modified the duration of exposure so that it ceases on postnatal day 10. The Agency chose this time for cessation of exposure for the following reasons: 1) while the revised period of exposure does not cover the entire period of lactation, it should still be sufficient to detect any potential effect that may be caused through exposure via breast milk, 2) any agent with a reasonable half-life would, theoretically, be eliminated sufficiently by the time testing begins (day 13 for motor activity) and would not significantly influence the results, and 3) while the nervous system is still undergoing some development beyond this time and, thus, effects on these events may be missed, the majority of the critical periods for CNS development have occurred by this time, most notably, proliferation of neuronal precursors in the cerebellum and hippocampus. Thus, it was felt that dosing through postnatal day 10 would maximize the detection of most developmental neurotoxic effects while minimizing the potential for pharmacologic influences of the agent on the outcome of functional evaluations.

4.COMMENT: The requirement for oral dosing by intubation is too rigid. Greater flexibility should be allowed for exposure via other routes.

EPA RESPONSE: The protocol has been revised to read: "The test substance or vehicle shall be administered orally. Other routes of administration may be acceptable, on a case-by-case basis, with ample justification/reasoning for this selection." The Agency, however, recognizes that conduct via other routes of exposure may necessitate modifications of the protocol because of potential problems with postnatal (lactational) exposure, that may be considered too drastic, and, thus, unacceptable. EPA is conducting research in this area and encourages others to do so, as well, in order to address the complications that may arise in studies conducted via routes other than oral.

5.COMMENT: The frequency of monitoring motor activity is excessive; furthermore, inclusion of two preweaning evaluations will separate the pups from the mother for lengths of time that may be detrimental to the pups.

EPA RESPONSE: In the proposal, EPA specified monitoring of motor activity on days 13, 17, 21, and day 60 ($\pm$ 2). These days were selected because they represent critical periods of motor development. Testing over a number of days provides the assessor

with information regarding the developmental pattern of motor activity. Interpretation of data from just a single day of testing would be much more difficult to interpret and less meaningful than more complete data on the ontogeny of motor activity and within-session habituation. Furthermore, the Agency does not have any reason to believe that handling of the pups, or separation from the mother for the length of time needed to carry out the motor activity testing, will result in any adverse effects on the pups; treated and control pups will be handled in the same manner to avoid any bias in the data.

6.COMMENT: A number of comments were made regarding the section on neuropathology. A suggestion was made to include simple morphometrics. In some cases, certain aspects of the Agency's procedures related to neuropathological evaluation were questioned. These included: 1) conducting neuropathology on animals sacrificed on postnatal day 4, 2) the number of animals included for neuropathologic evaluation, 3) plastic and paraffin embedding of tissue samples, 4) the qualitative examination, 5) the list of specified regions of the nervous system to be examined, 6) the semi-quantitative analysis (subjective diagnosis), and 7) the reporting of results.

EPA RESPONSE: The Agency has agreed to adopt the suggestion of including simple morphometrics. Detection of disruption of the development of the nervous system is the major purpose of this test. Compounds may alter nervous system development in a variety of ways including altering the rate and extent of growth of the nervous system. Alterations of this type are not always accompanied by gross neuropathological alterations. Thus, assessment of the extent of potential change in the normal state of development should be included. The approach will be to make simple measurements in regions known to be undergoing extensive growth at the time of sacrifice.

The Agency has formulated the following response to the questions raised regarding the aforementioned procedures:

1) The protocol has been revised to include neuropathological examination of animals sacrificed on postnatal day 11, the day after dosing ends, rather than postnatal day 4. This revision is based on several advantages to assessing the later time point. First, cumulative injury should be more apparent if exposure continues for an additional 6 days postnatally. Second, by postnatal day 11, several brain regions are approaching maximal proliferative activity (e.g., cerebellum). Third, a greater number of brain regions undergo further development during the additional 6 days, thus, the likelihood of revealing potential vulnerability is increased. Furthermore, brains of postnatal day 11 pups are more amenable to routine neuropathological analysis.

2) The requirement of 6 male and 6 female animals per dose group is to ensure an adequate sample size for statistical analysis of incidence and severity of neuropathological alterations as well as the morphometric analysis.

3) The goal of the procedures outlined for the preparation and processing of tissue samples is to optimally preserve tissue morphology for microscopic examination. The higher resolution obtainable in plastic embedded tissue is considered to optimize the detection of a number of types of lesions, particularly in the peripheral nervous system. In contrast, paraffin-embedded material is more amenable to sampling large regions of the nervous system and is considered optimal for a variety of special stains that may be useful in characterizing neuropathological alterations. Several organizations felt the requirement for separate animals for plastic and paraffin-embedding of tissue samples was excessive. Furthermore, commentors presented views that differences in plastic and paraffin techniques did not require the use of separate animals and that in an appropriate tiered evaluation scheme both plastic and paraffin-embedding could be used with tissue samples from the same animals. The requirement for separate animals is now eliminated, with paraffin being acceptable and plastic being recommended.

4) Many regions of the developing brain have been demonstrated to be sensitive to neurotoxic insult. The purpose of the qualitative examination is to identify regions within the developing brain that exhibit evidence of neuropathological alterations and to identify types of neuropathological alterations that result from exposure to the test substance. A stepwise evaluation is recommended since, if evidence of neuropathological alterations is not observed at the high dose level, additional processing of tissue samples is not required.

5) The Agency received some comments that the list of specified regions of the nervous system to be examined was inadequate, while others felt it was too detailed. Moreover, comments were received that argued that the list of potential types of neuropathological alterations also was too restricted. These lists were intended to serve as guidance. Since they appear to be subject to misinterpretation, the requirement for a thorough examination of the nervous system for any evidence of neuropathological alteration is now explicitly stated. However, it is noted that examination of the developing brain is particularly difficult and not usually the subject of routine neuropathological analysis. Therefore, a list of regions and the types of major alterations to be evaluated are included. In addition, a list of all structures examined is required in the final report. The requirement for examination of more than one section per region, however, has now been deleted.

6) The purpose of the semi-quantitative analysis is to evaluate the relationship between the incidence and severity of the neuropathological alterations and the exposure. Since the

rating scale is by necessity subjective, it is necessary to ensure that any bias resulting from the previous qualitative examination of the tissue samples is minimized. Several organizations commented that blind evaluation of tissue sections should not be required. In the initial qualitative analysis, in which the types of lesions and regions affected are first identified, blind evaluation is not required. However, in the semi-quantitative analysis in which the dose-response relationship is evaluated, it is imperative that the evaluation be as objective as possible. Moreover, since the semi-quantitative analysis focuses on a limited number of regions for lesions previously described in the qualitative analysis, blind reading is required to ensure objectivity. Thus, it is required that the subjective rating of the severity and incidence be performed without knowledge of treatment.

7) The data and analyses supplied in the report must be evaluated by Agency risk assessors. Thus, the report must be sufficiently detailed for the Agency to evaluate the quality of the study. Since no list of regions to be examined is outlined in the guideline, a list of regions examined must be supplied with the report. Similarly, an adequate description of lesions observed must be supplied also. The Agency received comments that the requirement of photomicrographs to document neuropathological alterations was extremely costly. The Agency has decided to recommend, rather than require, the use of photomicrographs to aid in the description of typical examples of treatment-related lesions.

7. COMMENT: Additional validation is needed before the glial fibrillary acidic protein (GFAP) radioimmunoassay should be included as part of the battery of tests in the developmental neurotoxicity study. Use of special stains should be at the discretion of the pathologist conducting the study.

EPA RESPONSE: Although, EPA believes that the GFAP assay has been shown to be sensitive to the neurotoxic effects of agents in both the adult and developing nervous systems, this assay has been deleted at this time. However, the Agency will continue using this assay experimentally and encourages others to do so, as well, in an effort to obtain additional validation of its use as a means to assess the neurotoxic potential of agents. In addition, if GFAP immunohistochemistry is used as a special stain in the neuropathology segment of the testing protocol and evidence of a glial response to toxicant injury is observed, application of the radioimmunoassay is encouraged in order to provide objective, quantitative dose-response data.

SCHEDULE-CONTROLLED OPERANT BEHAVIOR [1]

(a) Purpose. In the assessment and evaluation of the potential human health effects of substances, it may be necessary to test for functional neurotoxic effects. Substances that have been observed to produce neurotoxic signs in other toxicity studies (e.g., CNS depression or stimulation), as well as substances with a structural similarity to neurotoxicants affecting performance, learning, or memory may be appropriate to evaluate with this test. This guideline defines procedures for conducting studies of schedule-controlled operant behavior, one way of evaluating the rate and pattern of a class of learned behavior (Dews, 1972; NAS 1975, 1977, 1982). Our purpose is to evaluate the effects of acute and repeated exposures on the rate and pattern of responding under schedules of reinforcement. Any observed effects should be evaluated in the context of both the concordance between functional neurological and neuropathological effects and with respect to any other toxicological effects seen. Operant behavior tests may be also used to evaluate many other aspects of behavior (Laties, 1978). Additional tests may be necessary to completely assess the effects of any substance on learning, memory, or behavioral performance.

(b) Definitions.

(1) Neurotoxicity. Neurotoxicity is any adverse effect on the structure or function of the nervous system related to exposure to a chemical substance.

(2) Behavioral toxicity is any adverse change in the functioning of the organism with respect to its environment in relation to exposure to a chemical substance.

(3) Operant, operant behavior, operant conditioning. An operant is a class of behavioral responses which changes or operates on the environment in the same way. Operant behavior is further distinguished as behavior which is modified by its consequences. Operant conditioning is the experimental procedure used to modify some class of behavior by reinforcement or punishment.

(4) Schedule of reinforcement. A schedule of reinforcement specifies the relation between behavioral responses and the delivery of reinforcers, such as food or water (Ferster and Skinner, 1957). For example, a fixed ratio (FR) schedule requires a fixed number of responses to produce a reinforcer (e.g., FR 30). Under a fixed interval (FI) schedule, the first response after a fixed period of time is reinforced (e.g., FI 5 minutes).

(c) Principle of the test method. Experimental animals are trained to perform under a schedule of reinforcement and measurements of their operant behavior are made. Several doses of the test substance are then administered according to the experimental design (between groups or within subjects) and the duration of exposure (acute or repeated). Measurements of the

operant behavior are repeated. For use of this test to study learning, animals may be trained following exposure. A descriptive and statistical evaluation of the data is made to evaluate the nature and extent of any changes in behavior in relation to exposures to the test substance. Comparisons are made between any exposures that influence the behavior and exposures that have neuropathological effects or effects on other targets of the chemical.

(d) Test Procedures.

(1) Experimental design. These test procedures may be used to evaluate the behavior of experimental animals receiving either acute or repeated exposures. For acute exposure studies, either within-subject or between groups experimental designs may be used. For repeated exposure studies, between groups designs should be used, but within subject comparisons (pre-exposure and post-exposure) are recommended and encouraged.

(2) Animal selection.

(i) Species. For most studies the laboratory mouse or rat is recommended. Standard strains should be used. Under some circumstances other species may be recommended.

(ii) Age. Experimental animals should be young adults. Rats or mice should be at least 14 and 6 weeks old, respectively, prior to exposure.

(iii) Sex. Approximately equal numbers of male and female animals are required for each dose level and control group. Virgin females should be used.

(iv) Experimental history. Animals should be experimentally and chemically naive.

(3) Number of animals. Six to twelve animals should be exposed to each level of the test substance and/or control procedure.

(4) Control groups.

(i) A concurrent control group or control session(s) (according to the design of the study) are required. For control groups, subjects shall be treated in the same way as for an exposure group except that administration of the test substance is omitted.

(ii) Positive control data from the laboratory performing the testing shall provide evidence that the experimental procedures are sensitive to substances known to affect operant behavior. Both increases and decreases in response rate should be demonstrated. Data based on acute exposures will be adequate. Data shall be collected according to the same experimental design as that proposed for the test substance. Positive control data shall be collected at the time of the test study unless the laboratory can demonstrate the adequacy of historical data for this purpose, i.e., by the approach outlined in this guideline[2].

(5) Dose levels and dose selection. At least 3 doses

shall be used in addition to the vehicle control group (or sessions for within subject studies). Ideally, the data should be sufficient to produce a dose-effect curve. We strongly encourage the use of equally spaced doses and a rationale for dose selection that will maximally support detection of dose-effect relations.

(i) Acute studies. The high dose need not be greater than 2 g/Kg. Otherwise, the high dose shall result in significant neurotoxic effects or other clearly toxic effects, but not result in an incidence of fatalities that would preclude a meaningful evaluation of the data. The middle and low doses should be fractions of the high dose. The lowest dose shall produce minimal effects, e.g., an ED10, or alternatively, no effects.

(ii) Subchronic (and Chronic) Studies. The high dose need not be greater than 1g/Kg. Otherwise, the high dose shall result in significant neurotoxic effects or other clearly toxic effects, but not produce an incidence of fatalities that would prevent a meaningful evaluation of the data. The middle and low doses should be fractions of the high dose. The loweset dose shall produce minimal effects, e.g an ED10, or alternatively, no effects.

(6) Route of Exposure. Selection of route may be based on several criteria including, the most likely route of human exposure, bioavailability, the likelihood of observing effects, practical difficulties, and the likelihood of producing non-specific effects. For many materials, it should be recognized that more than one route of exposure may be important and that these criteria may conflict with one another. The route that best meets these criteria should be selected. Dietary feeding will be generally be acceptable for repeated exposure studies.

(7) Combined protocol. The tests described in this screening battery may be combined with any other toxicity study, as long as none of the requirements of either are violated by the combination.

(8) Study conduct. (i) Apparatus. Behavioral responses and the delivery of reinforcers shall be controlled and monitored by automated equipment located so that its operation does not provide unintended cues or otherwise interfere with the ongoing behavior. Individual chambers should be sound attenuated to prevent disruptions of behavior by external noise. The response manipulanda, feeders, and any stimulus devices should be tested before each session; these devices should periodically be calibrated.

(ii) Chamber assignment. Concurrent treatment groups should be balanced across chambers. Each subject should be tested in the chamber to which it is initially assigned.

(iii) Schedule of food availability. (A) If a non-preferred positive reinforcer is used, all subjects should be placed on a schedule of food availability until they reach a

fixed percentage e.g., 80 to 90 percent, of their ad libitum body weight, or kept at a fixed weight and fed after each session.

(B) Subjects must be trained until they display demonstrable stability in performance across days prior to exposure. One simple and useful criterion is a minimum number of sessions on the schedule and no systematic trend during the 5 days before exposure.

(iv) Time, frequency, and duration of testing.

(A) Time of testing. All experimental animals should be tested at the same time of day and with respect to the time of exposure. For acute studies, testing should be performed when effects are estimated to peak, which may be estimated from data on the functional observational battery, motor activity, or from pilot studies. For subchronic studies, subjects should be tested prior to daily exposure in order to assess cumulative effects.

(B) Frequency of testing. The maintenance of stable operant behavior normally will require regular and frequent (e.g., 5 days a week) testing sessions. Animals should be weighed on each test day.

(C) Duration of testing. Experimental sessions should be long enough to reasonably see the effects of exposure, but brief enough to be practical. Under most circumstances, a session length of 30-40 minutes should be adequate.

(v) Schedule selection. The schedule of reinforcement chosen should generate response rates that may increase or decrease as a function of exposure. Many schedules of reinforcement can do this: a single schedule maintaining a moderate response rate; fixed-interval schedules, which engender a variety of response rates in each interval; or multiple schedules, where different components may maintain high and low response rates.

(e) Data reporting and evaluation. The final test report must include the following information.

(1) Description of equipment and test methods. (i) A description of the experimental chambers, programming equipment, data collection devices, and environmental test conditions should be provided. Procedures for calibrating devices should also be described.

(ii) A description of the experimental design including procedures for balancing treatment groups, and the stability criterion should be provided.

(iii) Positive control data from the laboratory performing the test that demonstrates the sensitivity of the schedule used should be provided. Historical data may be used if all essential aspects of the experimental protocol are the same. Historical control data can be critical in the interpretation of study findings. We encourage submission of such data to

facilitate the rapid and complete review of the significance of effects seen.

(2) Results. (i) Data for each animal should be arranged by test group in tabular form including the animal identification number, body weight, pre-exposure rate and patterns of responding, changes in response rate and patterns produced by the chemical, and group data for the same variables, including standard measures of central tendency and variability e.g, means and standard deviations, and results of statistical analyses.

(3) Evaluation of data. (i) The findings should be evaluated in the context of preceding and/or concurrent toxicity studies and any correlated functional and histopathological findings. The evaluation shall include the relationship between the doses of the test substance and the incidence and magnitude of any observed effects, i.e. dose-effect curves for any effects seen.

(ii) The evaluation should include appropriate statistical analyses. Choice of analyses should consider tests appropriate to the experimental design, including repeated measures. There may be many acceptable ways to analyze data.

(iii) Any known citations from the open literature related to the interpretation of the neurotoxicity of the test material shall also be included.

(f) References. For additional background information on this test guideline the following references should be consulted.

Dews, P.B. "Assessing the Effects of Drugs", In Methods in Psychobiology, Vol. 2, Ed., R.D. Myers (New York: Academic Press, 1972) 83-124.

Ferster, C.B. Skinner, B.F. Schedules of Reinforcement. (New York: Appleton-Century-Crofts, 1957).

Laties, V.G. "How Operant Conditioning can Contribute to Behavioral Toxicology", Environmental Health Perspectives, 28:29-35 (1978).

National Academy of Science. Principles for Evaluating Chemicals in the Environment. (Washington, DC: National Academy of Sciences, 1975).

National Academy of Science. Principles and Procedures for Evaluating the Toxicity of Household Substances. (Washington, DC: National Academy of Sciences, 1977).

National Academy of Science. "Strategies to determine needs and priorities for toxicity testing." Appendix 3B. Reference Protocol Guidelines For Neurobehavioral Toxicity Tests. 2:123-129 (1982).

NOTES

1. This guideline has only been modified for clarity of prose and to make generic changes to conform to other guideline revisions in sections such as dose selection and route of administration. Otherwise, it is essentially identical to the guideline previously published by OTS in the Federal Register and codified in 40 CFR 798.6500

2. Positive control data need only be generated roughly once every year as long as most conditions remain the same.

PERIPHERAL NERVE FUNCTION[1]

(a) Purpose. In the assessment and evaluation of the potential human health effects of substances, it may be necessary to test for neurophysiological effects. Substances that have been shown to produce peripheral neuropathy in other neurotoxicity studies (or other neuropathological changes in peripheral nerves), as well as substances with a structural similarity to those causing such effects, may be appropriate to evaluate with this test. This guideline defines procedures for evaluating certain aspects of the neurophysiological functioning of peripheral nerves. Our purpose is to evaluate the effects of exposures on the velocity and amplitude of conduction of peripheral nerves. Any observed effects should be evaluated in the context of both the concordance between functional neurological and neuropathological effects and with respect to any other toxicological effects seen. Additional tests may be necessary to completely assess the neurophysiological effects of any substance.

(b) Definitions.

(1) Neurotoxicity. Neurotoxicity is any adverse effect on the structure or function of the nervous system related to exposure to a chemical substance.

(2) Conduction velocity is the speed at which the compound nerve action potential traverses a nerve.

(3) Amplitude is the voltage excursion recorded during the process of recording the compound nerve action potential. It is an indirect measure of the number of axons firing.

(c) Principle of the test method. The test substance is administered to several groups of experimental animals, one dose being used per group. The peripheral nerve conduction velocity and amplitude are assessed using electrophysiological techniques. The exposure levels at which significant neurotoxic effects are produced are compared to one another and to those levels that cause neuropathological effects and/or other toxic effects.

(d) Test Procedures.

(1) Animal selection. (i) Species and strain. Testing should be performed on a laboratory rodent unless such factors as the comparative metabolism of the chemical or species sensitivity to the toxic effects of the test substance, as evidenced by the results of other studies, dictate otherwise. All animals should have been laboratory-reared to ensure consistency of diet and environmental conditions across groups and should be of the same strain and from the same supplier. If this is not possible, groups shall be balanced to ensure that differences are not systemically related to treatment.

(ii) Age and weight. Young adult animals (at least 60 days for rats) must be used. Age (± 15 days for rats must not

vary across groups. Weights should be within ± 10 percent of the mean.

(iii) Sex. Either (or both) sex(es) may be used. Sex must not vary across groups.

(2) Number of animals. 20 animals should be exposed to each level of the test substance and/or control procedure. The goal is to be able to detect a 10 percent change from normal conduction velocity at the 5 percent level with 90 percent power.

(3) Control groups. (i) A concurrent control group is required. For control groups, subjects shall be treated in the same way as for an exposure group except that administration of the test substance is omitted.

(ii) Positive control data from the laboratory performing the testing shall provide evidence that the experimental procedures are sensitive to substances known to affect peripheral nerve function. Positive control data shall be collected at the time of the test study unless the laboratory can demonstrate the adequacy of historical data for this purpose, i.e., by the approach outlined in this guideline[2].

(4) Dose levels and dose selection. At least 3 doses shall be used in addition to the vehicle control group. Ideally, the data should be sufficient to produce a dose-effect curve. We strongly encourage the use of equally spaced doses and a rationale for dose selection that will maximally support detection of dose-effect relations.

(i) Acute studies. The high dose need not be greater than 2 g/Kg. Otherwise, the high dose shall result in significant neurotoxic effects or other clearly toxic effects, but not result in an incidence of fatalities that would preclude a meaningful evaluation of the data. The middle and low doses should be fractions of the high dose. The lowest dose shall produce minimal effects, e.g., an ED10, or alternatively, no effects.

(ii) Subchronic (and Chronic) Studies. The high dose need not be greater than 1g/Kg. Otherwise, the high dose shall result in significant neurotoxic effects or other clearly toxic effects, but not produce an incidence of fatalities that would prevent a meaningful evaluation of the data. The middle and low doses should be fractions of the high dose. The loweset dose shall produce minimal effects, e.g an ED10, or alternatively, no effects.

(5) Route of administration. Selection of route may be based on several criteria including, the most likely route of human exposure, bioavailability, the likelihood of observing effects, practical difficulties, and the likelihood of producing non-specific effects. For many materials, it should be recognized that more than one route of exposure may be important and that these criteria may conflict with one another. The route that best meets these criteria should be selected. Dietary feeding will be generally be acceptable for repeated exposure studies.

(6) Combined protocol. The test described in this guideline may be combined with any other toxicity study, as long as

none of the requirements of either are violated by the combination.

(7) Study conduct. (i) Choice of nerve(s). The nerve conduction velocity test must separately asses the properties of both sensory and motor nerve axons. Either a hind limb (e.g., tibial) or tail (e.g., ventral caudal) nerve must be chosen. Response amplitude may be measured in a mixed nerve.

(ii) Preparation. (A) In vivo testing of anesthetized animals is required. A barbiturate anesthetic is appropriate. Care should be taken to ensure that all animals are administered an equivalent dosage and that the dosage is not excessive. If dissection is used, extreme caution must be observed to avoid damage to either the nerve or the immediate vascular supply.

(B) Both core and nerve temperature must be monitored and kept constant (±0.5 °C) during the study. Monitoring of skin temperature is adequate if it can be demonstrated that the skin temperature reflects the nerve temperature in the preparation under use. Skin temperature should be monitored with a needle thermistor at a constant site, the midpoint of the nerve segment to be tested.

(C) Electrodes. (1) Choice of Electrodes. Electrodes for stimulation and recording may be made of any conventional electrode material, such as stainless steel, although electrodes made of non-polarizing materials are preferable. If surface electrodes are used, care must be taken to ensure that good electrical contact is achieved between the electrode and the tissue surface. Following each application, all electrodes must be thoroughly cleaned.

(2) Electrode placement. Electrode placement must be constant with respect to anatomical landmarks across animals (e.g., a fixed number of millimeters (mm) from the base of the tail). Distances between electrodes used to calculate conduction velocity must be measurable to ±0.5 mm. The recording electrodes should be as far from the stimulating electrodes as possible. A 40 mm separation is adequate in the caudal tail nerve of the rat.

(3) Recording conditions. The animal should be grounded at about the midpoint between the nearest stimulating and recording electrodes. With the preamplifier set at its maximal band width, the stimulus artifact should have returned to baseline before any neural response to be used in the analysis is recorded.

(D) The electrical stimulator must be isolated from ground. Biphasic or balanced pair stimuli to reduce polarization effects are acceptable. A constant current stimulator is preferred (and required for polarized electrodes) and should operate from about 10 uA to about 10 mA. If a constant voltage stimulator is used, it should operate to 250V. All equipment shall be calibrated with respect to time, voltage, and temperature.

(E) The recording environment should be enclosed in a Faraday cage unless electromagnetic field pickup can be shown to be more than 1.5 times the amplifier baseline noise, under recording conditions. The recording output should be amplified sufficiently to render the compound action potentially easily measurable with an oscilloscope. The amplifier should pass signals between 2.0 Hz and 4 kHz without more than a 3dB decrement. The preamplifier must be capacitatively coupled or, if direct coupled to the first stages, must be able to tolerate any DC potentials which the electrode-preparation interface produces, and operate without significant current leakage through the recording electrodes.

(F) A hard copy must be available for all waveforms or averaged waveforms from which measurements are derived, and for all control recording required by this standard. Hard copies must include a time and voltage calibration signal.

(iii) Procedure. (A) General. Stimulation should occur at an inter-stimulus interval significantly below the relative refractory period for the nerve under study. Stimulus intensity should be increased gradually until the response amplitude no longer increases. At this point the "maximal" stimulus current is determined. An intensity 25-50 percent (a fixed value in a given study) above the maximal intensity so determined should be used for determining response peak latency and response amplitude. Response peak latency may be read off the oscilloscope following single sweeps or determined by an average of a fired number of responsors. The baseline-to-peak height technique (Daube, 1980) is acceptable for determination of the nerve compound action potential amplitude, but in this case, at least 16 responses must be averaged.

(B) Motor nerve. Motor conduction velocity may be measured from a mixed nerve by recording the muscle action potential which follows the compound action potential of the nerve. The stimulus intensity should be adjusted so that the amplitude of the muscle action potential is supra-maximal. Measurement of the latency from stimulation to the onset of the compound muscle action potential gives a measure of the conduction time of the motor nerve fibers. To calculate the conduction velocity, the nerve must be stimulated sequentially in two places each with the same cathode-anode distance, and with the cathode located toward the recording electrode. The cathode to cathode distance between the two sets of stimulating electrodes should be divided by the difference between the two latencies of muscle action potential in order to obtain conduction velocity. Placement of electrodes shall be described; site of nerve stimulation may differ from point of entry through skin.

(C) Sensory nerve. The somatosensory evoked potential may be used to determine the sensory nerve conduction velocity in a mixed nerve. The cathode should be placed proximally at the two stimulation locations with the same cathode-anode distances. The recording electrodes are placed on the skull. The

conduction velocity is calculated by dividing the distance between the two stimulating cathodes by the difference between the two latencies of the largest primary peak of the somatosensory evoked potential. Between 64 and 123 responses should be averaged. The stimulation frequency should be about 0.5 Hz. Stimulus intensity should be the same as that used for determining the motor conduction velocity. Should the peak of the somatosensory response be so broad that it cannot be replicated with an accuracy of less than 5 percent of the latency difference observed, then a point on the rising phase of the potential should be chosen, e.g., at a voltage that is 50 percent of the peak voltage. Alternatively, the sensory nerve conduction velocity can be obtained from a purely sensory nerve or from stimulation of the dorsal rootlets of a mixed nerve, using two recording electrode pairs.

(e) Data collection, reporting and evaluation. The final test report must include the following information.

(1) Description of equipment and test methods. A description of the experimental chambers, programming equipment, data collection devices, and environmental test conditions should be provided.

(i) A description of the experimental design including procedures for balancing treatment groups should be provided.

(ii) Positive control data from the laboratory performing the test which demonstrate the sensitivity of the procedure being used should be provided. Historical data may be used if all essential aspects of the experimental protocol are the same. Historical control data can be critical in the interpretation of study findings. We encourage submission of such data to facilitate the rapid and complete review of the significance of effects seen.

(iii) Hard copies of waveforms from which measurements were made as well as control recordings should be included.

(iv) Voltage and time calibration referable to the standards of the Bureau of Standards or to other standards of accuracy sufficient for the measurements used should be included.

(v) Data demonstrating that nerve temperature was maintained constant throughout the recording period should also be included.

(2) Results. Data for each animal should be arranged by test group in tabular form including the animal identification number, body weight, nerve conduction velocity, and amplitude. Group summary data should also be reported, including standard measures of central tendency and variability, e.g., means and standard deviations, and results of statistical analyses.

(3) Evaluation of data. (i) The findings should be evaluated in the context of preceding and/or concurrent toxicity studies and any correlated functional and histopathological

findings. The evaluation shall include the relationship between the doses of the test substance and the incidence and magnitude of any observed effects, i.e. dose-effect curves for any effects seen.

(ii) The evaluation should include appropriate statistical analyses. Choice of analyses should consider tests appropriate to the experimental design, including repeated measures. There may be many acceptable ways to analyze data.

(iii) Any known citations from the open literature related to the interpretation of the neurotoxicity of the test material shall also be included.

(f) References. For additional background information on this test guideline the following references should be consulted:

Aminoff, M.J. (Ed.). Electrodiagnosis in Clinical Neurology. (New York: Churchill Livingstone, 1980).

Daube, J. "Nerve Conduction Studies," Electrodiagnosis in Clinical Neurology. Ed. M.J. Aminoff (New York: Churchill Livingstone, 1980). pp. 229-264.

Glatt, A.F., H.N. Talaat and W.P. Koella "Testing of peripheral nerve function in chronic experiments in rats," Pharmacology and Therapeutics, 5:539-534 (1979).

Johnson, E.W. Practical Electromyography. (Baltimore: Williams and Wilkins, 1980).

NOTES

1. This guideline has only been modified for clarity of prose and to make generic changes to conform to other guideline revisions in sections such as dose selection and route of administration. Otherwise, it is essentially identical to the guideline previously published by OTS in the Federal Register and codified in 40 CFR 798.6850.

2. Positive control data need only be generated roughly once every year as long as most conditions remain the same.

Assumptions

Oil price	$15.00/BBL
CO_2 price (fresh)	$ 0.80/MCF
CO_2 price (recycled)	$ 0.40/MCF
CO_2 utilization	10MCF/BBL
Well & Equipment Cost	$ 1.00/BBL
Workovers & Maintenance Cost	$ 1.50/BBL

Based on these assumptions, the following simplified economics can be generated.

Simple Economics of CO_2 Flooding ($/BBL)

	Case I	Case II
Gross Revenue	$15.00	$15.00
Less Royalties & Severance	$ 2.50	$ 2.50
Net Revenue	$12.50	$12.50
CO_2 Related Operating Cost		
CO_2	$ 8.00*	$ 6.60**
Wells/Equipment $ 1.00	$ 1.00	
Workovers/Maintenance	$ 1.50	$ 1.50
Subtotal	$10.50	$ 9.10
Operating Profit	$ 2.00/BBL	$ 3.40/BBL

* 100% fresh CO_2
** 65% fresh CO_2, 35% Recycled CO_2

A project with CO_2 and operating costs similar to these could be justified in today's environment with relatively low downside risk and good upside potential.

FUTURE POTENTIAL

The underlying domestic resource base which could be responsive to CO_2 flooding is in excess of 100 billion barrels of residual oil. The ultimate size of the potential is bounded more by economic criteria and competition from other EOR techniques than by technical limits.

Carbon dioxide has proven itself to be a highly versatile oil recovery agent, applicable to low permeability carbonate and dipping sandstones, useful as a substitute or enhancing agent of a waterflood or displacing agent of tertiary oil and may be used in either a miscible or immiscible mode. Generalized technical criteria for determining whether a reservoir is amenable to CO_2 flooding are of limited value and have lead to many potential CO_2 projects being overlooked. Individual reservoir analysis is required to establish oil displacement and efficiency. However, the following values have been established as desirable reservoir properties for miscible CO_2 flooding.

- Oil gravity greater than 25°API
- Oil viscosity less than 12 cp.
- Depth greater than 2500 feet
- Low vertical to horizontal permeability
- Multiple isolated and continuous pay intervals
- Reservoir dip to promote gravity stable displacement.

It has been demonstrated here and by the continuing interest in EOR that some CO_2 flooding can exist in today's environment. It is impossible to determine when oil prices will return to the point that CO_2 flooding will supply major quantities of oil. However, with 100 billion barrels of oil at stake, the future of CO_2 flooding is real.

WHAT IS THE NEED FOR ADDITIONAL SOURCES OF CARBON DIOXIDE

The existing sources of CO_2 can be divided into two categories, 1) natural sources of CO_2, and 2) manmade (industrial or by-product). The estimated reserves for the natural sources are listed below:

Natural Sources of CO_2

	SOURCE	RESERVES
1)	Sheep Mountain, S.E. Colorado	1 TCF
2)	Bravo Dome, N.E. New Mexico	6-12 TCF
3)	McElmo Dome, S. W. Colorado	10 TCF
4)	Jackson Dome, S.W. Mississippi	1- 3 TCF
5)	LaBarge-Big Piney, S.W. Wyoming	20 TCF
6)	Slanter-Brownfield, Central Utah	4 TCF
	Total	42-50 TCF

In many cases the determination of reserves is not applicable to manmade CO_2 as the quantity of CO_2 available is manufactured rather than produced from natural sources. The following are examples of major sources of manmade CO_2.

Sources of Manmade CO_2

- Gas processing plants, eg. Val Verde Basin, Texas
- Fertilizer plants, eg. Enid, Oklahoma
- Ammonia Plant, Skillington, Louisiana
- Coal gasification, eg. Great Plains, N. Dakota
- Refinery hydrogenation units, California, Texas, Louisiana

The National Petroleum Council study of enhanced oil recovery estimated that 5.5 billion barrels of oil could be economically recovered at $30.00 per barrel using current CO_2 technology.

In their view, higher oil prices ($50.00 per barrel) would add 2.2 billion and advanced technology would add less than a billion as tabulated below:

NPC Estimates of Economic Oil Recovery from CO_2 Flooding (Billions of Barrels)

Nominal Crude Oil Price ($/BBL)	Current Technology	Advanced Technology
30	5.5	6.1
40	7.0	7.8
50	7.7	8.5

Similar studies have estimated the ultimate economic recovery from CO_2 floods at $30 per barrel to approach 10 billion barrels of oil. The amount of CO_2 required to recover this 5.5 billion to 10 billion barrels of oil would be between 55 TCF and 100 TCF. Comparing this to the known natural CO_2 reserves would indicate that between 5 TCF and 50 TCF of manmade CO_2 would be required at a $ 30 oil price.

SUMMARY

1) Limited CO_2 flooding can exist and develop under the current $15-18/BBL environment, however, for large scale CO_2 flooding to exist, oil prices will have to rise considerably.

2) The long term outlook for CO_2 flooding is bright due to the relative large quantities of additional oil which could be produced from existing reservoirs and the inevitable long term rise of oil prices.

3) The known reserves of natural CO_2 will be insufficient to supply the long term needs of the CO_2 industry. Additional sources of manmade CO_2 will have to be developed.

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CURRENT STATUS OF THE USE OF CO_2 FOR ENHANCED OIL RECOVERY

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ABSTRACT

Carbon dioxide flooding is probably the fastest growing enhanced oil recovery method in use today with about 100 projects underway or planned. There are both technical and economic reasons for this steady growth. Large volumes of CO_2 are now available in some areas at high purity and at pressures needed for efficient oil recovery. Existing pipelines are already capable of delivering large quantities of CO_2 to reservoirs which respond well to CO_2 flooding. If pressure requirements are met, the displacement of oil by CO_2 can be efficient, at least in those areas of the oil reservoirs swept by CO_2. Although CO_2 bypasses some oil, and breaks through early at the production wells, oil is produced effectively for a very long period. Fortunately, the produced CO_2 can be separated and recycled efficiently to achieve good ultimate recovery. In general, as CO_2 floods in the Permian Basin of west Texas and eastern New Mexico mature, it appears that the net oil recovery will be even better than predicted. The technology of CO_2 flooding is still evolving, and the economics depend strongly on crude oil prices. The current status of CO_2 flooding is described with reference to specific field results. Information on all of the major CO_2 floods in the United States is given, and the projects are located on maps.

INTRODUCTION

At the 1985 and 1986 International Energy Agency (IEA) Workshops on EOR in Tokyo, Japan, and Hannover, Germany, it was reported that gas injection was one of the faster growing enhanced recovery methods, and that CO_2 flooding was becoming the most important gas injection method in the United States.[1,2] These facts are still true today. However, even though the number of gas injection projects is increasing steadily, the rate of increase in CO_2 flooding is leveling off for the first time since 1980. Fig. 1 shows the trends for all of the active enhanced recovery projects in the United States, and Fig. 2 shows the gas injection projects. It can be seen from Fig. 2 that the number of CO_2 projects is still increasing at a rate of about eight projects per year even though the recent drop in oil prices will probably slow this rate of increase. However, the most recent Oil and Gas Journal survey on EOR reports that 42 new CO_2 projects are planned to start before the end of 1988[3], so the increase in oil recovery from CO_2 floods will certainly continue until well into the next century.

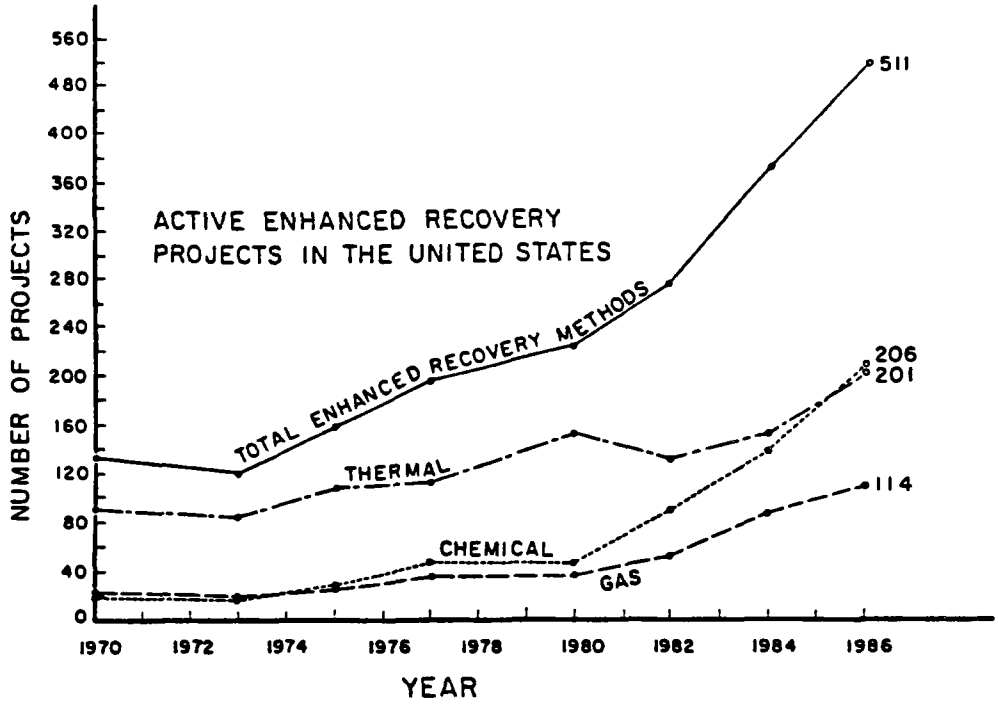

Figure 1. Enhanced Oil Recovery Trends in the United States Since 1970. (Does Not Include 107 Planned for 1986-88.) (After Reference 2.)

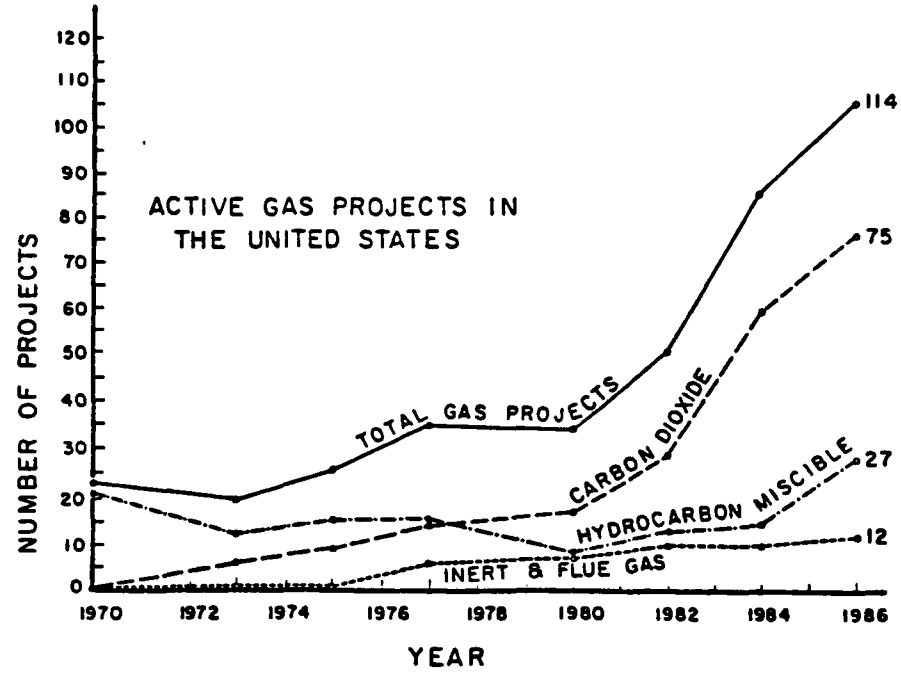

Figure 2. Increase of Gas Injection Enhanced Recovery Projects in the United States. (Does Not Include 42 CO_2 Projects Planned for 1986-88.) (After Reference 2.)

The economic and technical reasons for the growth in CO_2 flooding have been reported by a number of authors.[4-11] At present, reservoir characteristics and the availability of CO_2 favor three general areas in the United States which are shown in Fig. 3. These are the Permian Basin of west Texas and eastern New Mexico, Mississippi and the Gulf Coast Area, and the Wyoming-Colorado-Utah area.

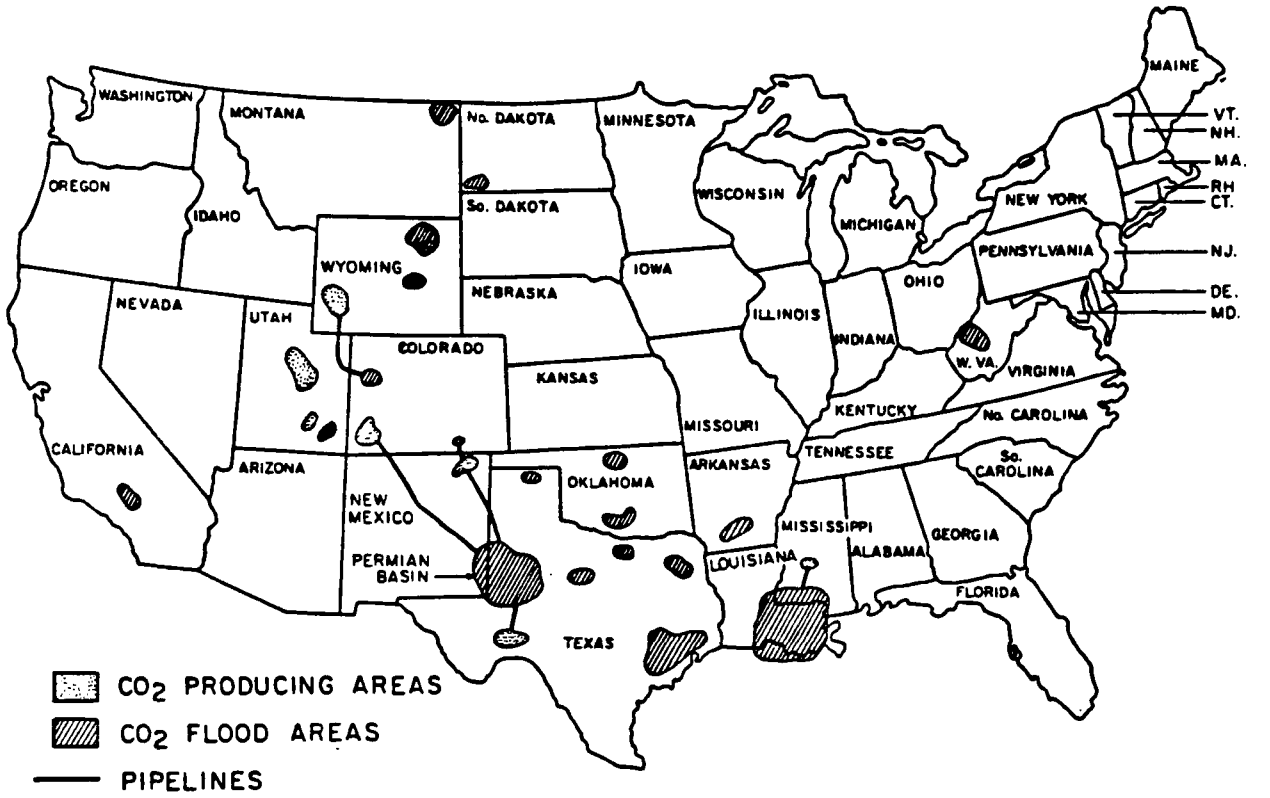

Figure 3. General Locations of CO_2 Flooding Areas and Natural Sources in the united States.

Current Sources of CO_2 for Enhanced Oil Recovery

The most widely publicized of the active CO_2 flooding areas has been the Permian Basin because of the availability of CO_2 within reasonable pipeline distance of the oil reservoirs. Recently, however, the La Barge-Big Piney area of western Wyoming has received attention. It has been reported that there are 20-25 trillion cubic feet (TCF) of CO_2 in the Wyoming sources. This approximately equals the combined sources of Sheep Mountain, Colorado (about 2 TCF), the McElmo Dome of southwest Colorado (about 10 TCF), and the Bravo Dome of northeastern New Mexico (also containing 10 TCF) of CO_2 reserves. Thus, in the Rocky Mountain area, there are in excess of 45 TCF of CO_2 available from natural sources. While the Wyoming CO_2 resource consists of approximately 65% CO_2 plus methane and other hydrocarbons of low molecular weight, the Permian Basin sources (see Fig. 4) produce an injection gas which has a much higher concentration of CO_2. The McElmo Dome and Sheep Mountain sources in southern Colorado contain small amounts of hydrocarbons, whereas the Bravo Dome reservoir in northeastern New Mexico contains CO_2 of more than 99% purity.

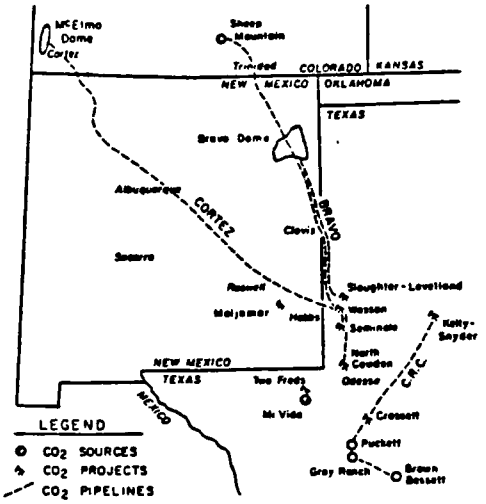

Figure 4. CO_2 Pipelines Which Supply the Permian Basin.

Fig. 4 illustrates three pipelines which deliver CO_2 to the carbonate reservoirs of the Permian Basin. Because these pipelines deliver CO_2 to the oilfields at a reasonable cost, half of the 53 miscible projects listed in Table 1-M are in this Permian Basin area. The current capacities of the pipelines are as follows: Sheep Mountain (completed in 1983) 500 million cubic feet (MMCF) per day; the Cortez Pipeline (completed in 1984) 650 MMCF per day, and is capable of almost one billion cubic feet (BCF) per day; the Bravo Pipeline (completed in 1985) from the Bravo Dome to the Permian Basin is capable of delivering from 400-700 MMCF per day. The National Petroleum Council (NPC)[8] forecasts that the Rocky Mountain and Permian Basin areas would require about three BCF of CO_2 per day to reach the enhanced recovery target of 500,000 to 600,000 barrels of oil per day by the year 2000. These forecasts appear to be quite reasonable; the current CO_2 pipeline capacity will be close to three BCF per day when the Wyoming-Colorado and other pipelines which are now in the final planning or construction stages are completed. The Exxon-Chevron pipeline (200 MMCF/D) which supplies the Rangely, Colorado, CO_2 flood is illustrated in Fig. 4a.

In addition to the pipelines which supply CO_2 for EOR in the Rocky Mountain and Permian Basin areas, Fig. 5 shows the Choctaw Pipeline which is, or will be servicing, several CO_2 floods in Mississippi and Louisiana.[12] The Jackson Dome CO_2 source, northeast of Jackson, Mississippi, has several deep reservoirs (14,000-16,000 feet) which contain 6 TCF of high-purity CO_2. The reservoirs closest to the Jackson Dome igneous intrusion contain CO_2 of 99% purity. The purity falls off (more light hydrocarbons are present) at distances greater than 25 miles northeast of the Jackson Dome. The Mississippi section of the Choctaw Pipeline (Fig. 5) is completed and supplying CO_2 at high pressures to the expanding Little Creek CO_2 flood. Until it crosses the Mississippi River, it is a 20 inch, carbon steel pipeline; from there to Weeks Island, it is a 10 inch line, and this section should be completed by the fall of 1987. In addition to the ongoing CO_2 floods at

Little Creek and Weeks Island, Shell is planning CO_2 recovery projects for the Mallalieu, Olive, and White Castle fields shown on Fig. 5.[12]

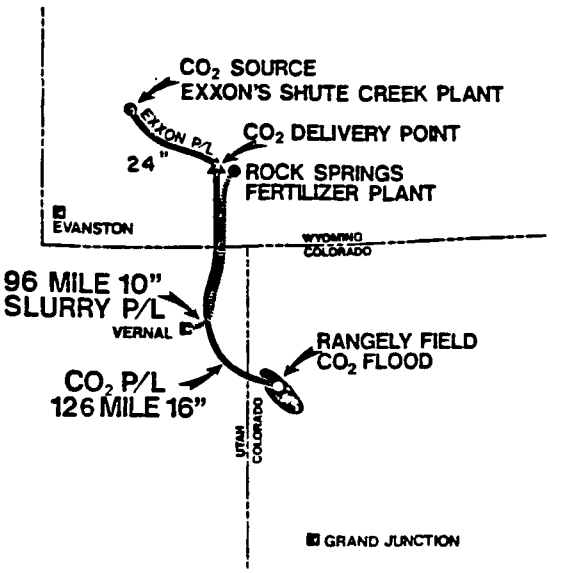

Figure 4a. CO_2 Supply for Rangely Field in Colorado. (Courtesy of S.L. Walker, Chevron, USA, Inc.)

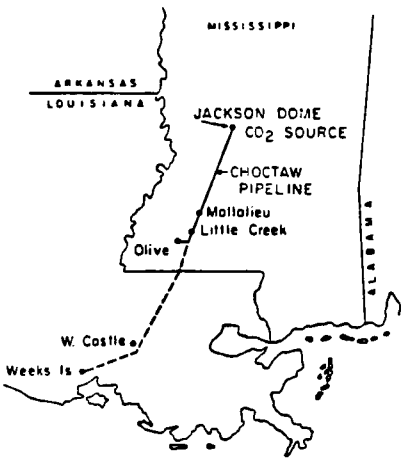

Figure 5. The Choctaw CO_2 Pipeline for Enhanced Oil Recovery Projects in Mississippi and Louisiana. (After Reference 12.)

A few CO_2 floods are supplied by CO_2 from industrial sources. For example, East Velma and Northeast Purdy (project nos. 6 and 25 in Table 1-M) are supplied by a fertilizer plant, while Lick Creek (project no. 7 in Table 1-IM) receives CO_2 from an ammonia plant.

Table 1-M. Miscible CO_2 Projects in the USA

(Fields A-N)

Project No.	Field Name	State	County	Operator	Start date	Area, acres	Number Wells Prod.	Number Wells Inj.	Pay zone	Lithology	Porosity %	Permeability md.
1	Alvord South Field	Tex.	Wise	Mitchell Energy	1980	2,291	245	10	Caddo	Congl.	12.8	55
2	Bay St. Elaine	La.	Terrebonne	Texaco	1/81	9	2	1	8000-Foot	S	32.9	1,480
3	Crossett	Tex.	Crane & Upton	Shell Western E&P	4/72	1,500	23	11	Devonian	Tripolitic chert	22.0	5
4	Dillinger Ranch	Wyo.	Campbell	Tenneco	10/80	600	20	10	Minnelusa Sand	S	13	10-100
5	Dollarhide	Tex.	Andrews	Unocal	5/85	6,183	62	43	Devonian	Dolo./ Tripolite	13.5	17
6	East Velma	Okla.	Stephens	Arco	1983				Sims	S	17	70
7	Farnsworth, North	Tex.	Ochiltree	Dorchester Enhanced Co.	6/80	1,472	8	6	Marmaton "B"	LS	12	41
8	Ford Geraldine	Tex.	Reeves & Culberson	Conoco	2/81	3,850	198	123	Delaware	S	23.0	64
9	Garber	Okla.	Garfield	Arco	10/81	80	9	4	Crews	S	19.0	12
10	Granny's Creek	W.Va.	Clay	Columbia Gas Transmission	6/76	7	4	1	Pocono Big Injun	S	16	7
11	Greater Aneth	Utah	San Juan	Superior	1982	13,357	140	21	Aneth	LS	10	
12	Kurten	Tex.	Brazos	Chevron	8/81	672	5	4	Woodbine	S	12.0	0
13	Levelland Unit	Tex.	Hockley	Amoco	3/73	13	2	6	San Andres	Dolo.	11.5	4
14	Levelland (Mini Test)	Tex.	Hockley	Amoco	8/78	1.5	1	4	San Andres	Dolo.	11.8	4
15	Little Creek Pilot	Miss.	Lincoln & Pike	Shell	2/74	31	3	1	Tuscaloosa	S	23	33
16	Little Creek Field	Miss.	Lincoln & Pike	Shell Western E&P	12/85	8,200	110	40	Lower Tuscaloosa	S	23.0	33
17	Little Knife	N.Dak.	Billings	Gulf	1/81	5	4	1	Madison Canyon Zone D	L/Dolo.	18	22
18	Maljamar Pilot MCA Unit	N.M.	Lea	Conoco	5/83	5	4	1	Grayburg/ San Andres	S/LS	11.0	18
19	McElmo Creek Unit	Utah	San Juan	Mobil	2/85	13,440	170	100	Ismay Desert Creek	LS	14.0	5
20	McElroy	Tex.	Upton	Southland Royalty	2/81	640	38	20	San Andres	Dolo.	11.6	2
21	Mead Strawn	Tex.	Jones	Union	12/64	43	3	4	Strawn	S	11	12
22	Means (San Andres)	Tex.	Andrews	Exxon	11/83	6,700	248	176	San Andres	LS	9.0	20
23	North Coles Levee	Calif.	Kern	Arco	6/81	70	8	3	Stevens	S	19.5	9
24	North Cowden Unit	Tex.	Ector	Amoco	2/79	12	2	6	Grayburg	Dolo/LS	10.0	5
25	Northeast Purdy	Okla.	Garvin	Cities Service	9/82	8,320	106	102	Springer	S	13.0	44

Abbreviations: JS = Just started; HF = Half finished; NC = Near completion; Term. = Terminated; TETT = Too early to tell; Prom. = Promising; Succ. = Successful; Disc. = Discouraging

Table 1-M. Miscible CO_2 Projects in the USA (cont'd.)

(Fields A-N -- data continued)

Project No.	Field Name	Depth ft.	Reservoir oil API Gravity	Viscosity cp	@ F	Previous Prodn.	Residual oil saturation % Start	End	Project maturity	Total prodn. bo/d	Enhanced prodn. bo/d	Project eval.	Profit
1	Alvord South Field	5,700	44.0	0.39	154	WF	60.0	45.0	HF	680	200	Prom.	
2	Bay St. Elaine	7,400	36.0	0.67	170	Prim.	20.0	5.0	NC	7	7	Disc.	No
3	Crossett	5,300	44.0	0.36	106	Gas inj'n	34.0	22.0	HF	2,000	2,000	Succ.	Yes
4	Dillinger Ranch	9,000	37.0	0.86	230	Prim/WF			HF	120	60		
5	Dollarhide	8,000	40.0	0.44	122	Prim/WF	35.0	22.0	JS	1,900		TETT	TETT
6	East Velma		26.0	2.50		Prim/WF			JS				
7	Farnsworth, North	6,500	39	1.61	131	Prim.	56		HF				
8	Ford Geraldine	2,680	40.0	1.40	83	Prim/WF			JS	395	395	Succ.	No
9	Garber	1,900	44.0	1	100	WF	30.0	16.0	NC	35		Succ.	Yes
10	Granny's Creek	2,000	45.0	3.14	75	Prim/WF			Term.				
11	Greater Aneth	5,750	42.0	0.47	135	Prim/WF	43						
12	Kurten	8,300	38.0	0.40	230	Prim.	40.0		JS	160	120		
13	Levelland Unit	4,900	30.0	2.30	105	WF	74.0		HF	47	21	TETT	
14	Levelland (Mini Test)	4,900	30.0	2.30	105	WF	43.0		HF	9	6	TETT	
15	Little Creek Pilot	10,700	39	0.40	248	Prim/WF	21		Term.			Succ.	
16	Little Creek Field	10,640	38.0	0.40	248	WF	21.0	2.0	JS	3,300	3,300	Prom.	TETT
17	Little Knife	9,800	43.0	0.20	240	Prim.							
18	Maljamar Pilot HCA Unit	3,665	36.0	0.80	90	Prim/WF			NC	50	50		
19	McElmo Creek Unit	5,600	41.0	0.50	125	Prim/WF	50.0		JS	5,600		TETT	TETT
20	McElroy	3,850	31.0	2.30	86	WF			Term.				
21	Mead Strawn	4,475	41	1.30	135	Prim.	39		Term.			Succ.	
22	Means (San Andres)	4,300	29.0	6.00	97	WF			JS				
23	North Coles Levee	9,000	36.0	0.45	235	Prim.	34.0	25.8	HF	150		Disc.	No
24	North Cowden Unit	4,300	34.0	1.67	94	WF	46		JS	22		TETT	
25	Northeast Purdy	9,400	38.0	1.20	148	WF	46.0	40.0	NC	3,500	950	TETT	TETT

Table 1-M. Miscible CO_2 Projects in the USA (cont'd.)

(Fields P-W)

Project No.	Field Name.	State	County	Operator	Start date	Area, acres	Number Wells Prod.	Number Wells Inj.	Pay zone	Lithology	Porosity %	Permeability md.
26	Paradis (No. 8)	La.	St. Charles	Texaco	2/82	320	7	3	No. 8	S	27.0	795
27	Paradis	La.	St. Charles	Texaco	2/82	347	12	4	Lower 9000-Foot	S	26.0	770
28	Pittsburg	Tex.	Camp	Chevron	6/85	43	4	1	Pittsburg	Limey Sand	11.0	2
29	Quarantine Bay	La.	Plaquemines	Chevron	10/81	57	4	1	4 Sand Reservoir	S	30.0	100-1,000
30	Rangely	Colo.	Rio Blanco	Chevron	10/86	20,000	360	360	Weber	S	15	20
31	Rankin	Tex.	Harris	Petromac Inc.	1/81	80	6	1	Yegua	S	27.0	300
32	Raymond	Mont.	Sheridan	Santa Fe Energy	8/83	685	2	1	Nisku	LS	8.2	13
33	Rock Creek	W.Va.	Roane	Pennzoil	11/76	20	2	6	Big Injun	S	22	20
34	Rose City North	Tex.	Orange	Highland Resource	4/81	800	9	5	Hackberry	S	37.0	4,500
35	Sable	Tex.	Yoakum	Arco	3/84	825	31	11	San Andres	Dolo.	8.4	2
36	SACROC Unit	Tex.	Scurry	Chevron	1/72	49,900	887	379	Canyon Reef	LS	3.9	19
37	Seminole	Tex.	Gaines	Amerada Hess	4/83	15,700	328	133	San Andres	LS	13	
38	Sho-Vel-Tum	Okla.	Stephens	Arco	9/82	1,100	65	43	Sims	S	16.0	70
39	Slaughter Estate	Tex.	Hockley	Amoco	11/72	12	2	6	San Andres	Dolo.	10.5	4
40	Slaughter (Frazier Unit)	Tex.	Hockley	Amoco	12/84	1,600	64	37	San Andres	Dolo/LS	10.0	4
41	Slaughter - (Central Hallet Unit)	Tex.	Hockley	Amoco	12/84	5,700	325	73	San Andres	Dolo/LS	13.0	7
42	S.Bishop Ranch (9200')	Wyo.	Campbell	Grace Petroleum	1/82	640	5	2	Minnelusa	S	16.0	50
43	S.Bishop Ranch (9400')	Wyo.	Campbell	Grace Petroleum	1/82	1,280	5	4	Minnelusa	S	15.0	150
44	Tinsley	Miss.	Yazoo	Pennzoil	11/81	1,338	21		Perry	S	26.4	49
45	Twofreds	Tex.	Loving, Ward & Reeves	HNG Fossil Fuels	1/74	4,392	42	33	Delaware	S	19.5	32
46	University Waddell	Tex.	Crane	Chevron	5/83	920	50	13	Devonian	Dolo.	12.0	14
47	Vacuum	N.M.	Lea	Phillips	2/81	4,900	237	97	San Andres	Dolo.	11.7	11
48	Wasson (Denver Unit)	Tex.	Yoakum	Shell Western E&P	4/83	20,000	840	280	San Andres	Dolo.	12.0	8
49	Wasson (ODC Unit)	Tex.	Yoakum	Amoco	12/84	7,800	316	250	San Andres	Dolo/LS	9.0	5
50	Weeks Island Field	La.	New Iberia	Shell Western E&P	1979	8	2	1	S RES B	S	26.0	1,800
51	Welch	Tex.	Dawson	Cities Service	2/82	2,675	129	132	San Andres	LS	9.3	9
52	Wellman	Tex.	Terry	Union Texas Petro	7/82	1,400	29	2	Wolfcamp	LS	4.2	100
53	West Sussex Unit	Wyo.	Johnson	Conoco	12/82	10	3	1	Shannon	S	19.5	121

Table 1-M. Miscible CO_2 Projects in the USA (cont'd.)

(Fields P-W -- data continued)

Project No.	Field Name	Depth ft.	Reservoir oil API Gravity	Viscosity cp	@ F	Previous Prodn.	Residual oil saturation % Start	End	Project maturity	Total prodn. bo/d	Enhanced prodn. bo/d	Project eval.	Profit
26	Paradis (No. 8)	8,600	39.0	0.40	192	Prim.	3.0	2.0	HF	400	400	Prom.	No
27	Paradis	10,400	37.0	0.50	205	Prim.	62.0	48.0	HF	575	575	Prom.	No
28	Pittsburg	8,000	41.0		205	WF			NC	237	105	Succ.	Yes
29	Quarantine Bay	8,120	32.0	0.99	183	Prim.	55.0		HF	85	85	Prom.	No
30	Rangely	6,000	34.0		160	WF	36.0		JS	30,000		TETT	TETT
31	Rankin	7,900	37.0	0.60	192	WF	55.0	25.0	JS	80	80	Prom.	No
32	Raymond	7,900	40.0	0.40	178	Prim.			JS	86	46	Prom.	Yes
33	Rock Creek	2,000	43	3.20	73	Prim/			NC				
34	Rose City North	8,200	37.0	2.00	180	WF	50.0	35.0	HF	460	460	Prom.	No
35	Sable	5,200	32.0	1.46	107	WF			JS	750		TETT	TETT
36	SACROC Unit	6,700	41.0	0.35	130	Prim/WF	55.0	25.0	NC	43,863	16,000	Succ.	Yes
37	Seminole	5,300	35	1.70	105	Prim/WF	54			41,800		Prom.	
38	Sho-Vel-Tum	6,200	25.0	3.30	115	WF	59.0	42.0	JS	2,500	750	Prom.	Yes
39	Slaughter Estate	4,950	27.0	2.00	105	WF	61.0		NC	28	28	Succ.	
40	Slaughter (Frazier Unit)	4,950	31.0	1.40	105	WF	42.0		JS	3,000		Prom.	
41	Slaughter - (Central Mallet Unit)	4,950	31.0	1.40	105	WF	65.0		JS	7,500		Prom.	
42	S.Bishop Ranch (9200')	9,200	35.0	1.14	220	WF			JS				
43	S.Bishop Ranch (9400')	9,400	34.0		180	WF			JS				
44	Tinsley	4,800	39.0	1.50	175	WF	65.0	38.0	HF	400	400	Prom:	No
45	Twofreds	4,900	36.0	1.50	105	WF			HF	892	892		
46	University Waddell	8,500	43.0	0.45	140	WF	71.0		JS	2,000	70	TETT	Yes
47	Vacuum	4,500	38.0	1	101	Prim.	70.0	50.0	JS	12,400	80	TETT	TETT
48	Wasson (Denver Unit)	5,200	33.0	1.30	105	WF	40.0	27.0	JS	46,000	1,200	Succ.	Yes
49	Wasson (ODC Unit)	5,100	32.0	1.30	110	WF	45.0		JS	13,000		Prom.	
50	Weeks Island Field	12,760	32.0	0.50	225	WF	22.0	2.0	NC	160	160	Succ.	No
51	Welch	4,890	34.0	2.15	96	WF	30.0	18.0	HF	3,100	300	Succ.	No
52	Wellman	9,800	43.5	0.54	151	WF	35.0	10.0	JS	7,000		TETT	TETT
53	West Sussex Unit	3,040	38.0	1.70	93				Term.	79	79		

Table 1-IM. Immiscible CO_2 Projects in the USA

Project No.	Field	State	County	Operator	Start date	Area, acres	Number Wells Prodn.	Number Wells Inj.	Pay zone	Lithology	Porosity %	Permeability md.
1	Bayou Sale	La.	St. Mary	Texaco	5/84	564	5	2	St. Mary	S	31.0	500
2	Cote Blanche Bay West	La.	St. Mary	Texaco	3/84	55	3	3	14 Sand	S	29.0	322
3	East Coyote-Hualde Dome Units	Calif.	Orange	Unocal	6/82	766	92	21	1,2,3 Anaheim	S	26.0	400
4	Heidelberg	Miss.	Jasper	Chevron	12/83	40	1		Eutaw	S	25.0	74
5	Lafitte	La.	Jefferson	Texaco	8/84	271	5	2	8900-Foot	S	27.0	250
6	Lake Barre	La.	Terrebonne	Texaco	3/84	1,164	12	4	Upper M	S	25.0	139
7	Lick Creek	Ark.	Bradley-Union	Phillips	1/76	1,640	39	13	Meakin	S	30.3	1,200
8	Magnet Withers BH&S State Tracts	STex.	Wharton	Texaco	10/83	1,224			Magnet Withers	S	23.0	1,700
9	Magnet Withers Pierce Estates B&C	Tex.	Wharton	Texaco	7/83	500				S	23.0	1,700
10	Manvel	Tex.	Brazoria	Texaco	11/83	128			Oligocene	S	30.0	1,000
11	Manvel	Tex.	Brazoria	Texaco	10/82	43			Oakville	S	30.0	400
12	Paradis	La.	St. Charles	Texaco	3/84	110	2	2	Main Pay	S	28.0	1,033
13	Pewitt Ranch	Tex.	Titus	Exxon	6/83		1		Paluxy	S	24.0	1000-1500
14	Pickett Ridge	Tex.	Wharton	Texaco	5/83	726			Pickett Ridge	S	30.0	1,200
15	Pierce Ranch	Tex.	Wharton	Texaco	1/83	480			Pierce Ranch	S	31.8	534
16	Pittsburg	Tex.	Camp	Chevron	11/83	120	3		Sub-Clarksville	S	23.0	460
17	Plymouth	Tex.	San Patricio	Texaco	10/83	380			Main Greta	S	31.0	350
18	Sho-Vel-Tum	Okla.	Stevens	Texaco	5/83	120	2		Deese	S	13.0	100
19	South Marsh Island Block 6	La.	Offshore	Texaco	6/85	82	2	1	Rob E-S	S	29.6	323
20	Talco	Tex.	Franklin	Texaco	5/82	240			Paluxy	S	25.0	388
21	Thompson	Tex.	Fort Bend	Texaco	1/83	100			Frio	S	27.0	100-1,000
22	West Columbia	Tex.	Brazoria	Texaco	6/83	33			PTSD	S	30.0	560
23	West Delta Block 109	La.	Offshore	Texaco	6/85	48	1	1	10200-Foot	S	27.0	205
24	West Delta Block 109	La.	Offshore	Texaco	6/85	74	1	1	10800-Foot	S	30.0	1,900
25	West Delta Block 109	La.	Offshore	Texaco	6/85	75	1	1	13100-Foot	S	29.7	68
26	West Delta Block 109	La.	Offshore	Texaco	6/85	78	1	1	12500-Foot	S	27.0	1,032
27	Wilmington	Calif.	Los Angeles	Champlin	2/84	156	10	12	Tar	S	24.0	465
28	Wilmington	Calif.	Los Angeles	Champlin	3/81	41	3	4	Tar	S	24.0	465
29	Withers North	Tex.	Wharton	Texaco	3/83	454			Withers N.	S	25.0	1,050
30	Withers North	Tex.	Wharton	Texaco	5/83	768			C-Sand	S	25.0	400
31	Yates	Tex.	Pecos & Crockett	Marathon Oil	11/85	14,300	895	13	Grayburg/ San Andres	Dolo.	17.0	175

Table 1-IM. Immiscible CO_2 Projects in the USA (cont'd.)

Project No.	Field	Depth ft.	Reservoir oil API Gravity	Reservoir oil Viscosity cp	@ F	Previous Prodn.	Residual oil saturation % Start	Residual oil saturation % End	Project maturity	Total prodn. bo/d	Enhanced prodn. bo/d	Project eval.	Profit
1	Bayou Sale	10,000	34.0	0.4	194	Prim.	50.0	45.0	JS	2,000	30	Succ.	Yes
2	Cote Blanche Bay West	8,000	32.0	1.3	184	WF	50.0	45.0	JS	80	10	Prom.	Yes
3	East Coyote-Hualde Dome Units	3300-460	23.0	12	130	WF			Term.	1,600		Disc.	No
4	Heidelberg	5,060	20.0	15	150	Prim.			HF				
5	Lafitte	8,900	34.0	0.7	185	Prim.	50.0	45.0	JS	400	30	Succ.	Yes
6	Lake Barre	13,000	33.0	0.4	236	WF	50.0	45.0	JS	650	50	Succ.	Yes
7	Lick Creek	1100-170	17.0	160	118	Prim.	55.0	46.0	NC	450	400	Succ.	Yes
8	Magnet Withers BH&S State Tracts	5,500	26.0	2.3	154	Gas inj'n	35.0	31.0	JS	563	5	Succ.	Yes
9	Magnet Withers Pierce Estates B&C	5,500	26.0	2.3	154	Gas inj'n	35.0	34.0	JS	200	17	Succ.	Yes
10	Manvel	5,000	26.7	7.2	149	Prim.	45.0	20.0	JS	550	30	Succ.	Yes
11	Manvel	4,000	25.0	4.4	149	Prim.	42-65	Variable	JS	127			
12	Paradis	10,200	38.0	0.5	195	WF	50.0	45.0	JS	250	15	Succ.	Yes
13	Pewitt Ranch	4,500	19.0	30	160	Prim.			JS				
14	Pickett Ridge	4,600	25.0	2.5	138	Prim.	29.0	28.0	HF	112			No
15	Pierce Ranch	4,900	24.4	4.57	155	Prim.	48.0	18.0	HF	429	1	Disc.	No
16	Pittsburg	3,800	14.0	2,200	120	Prim.			JS				
17	Plymouth	4,650	23.3	3.19	150	Prim.	31.5	20.0	Term.	150		No	
18	Sho-Vel-Tum	5,530	24.0	18	129	Prim.	49.0	40.0	Term.	12	8	Prom.	Yes
19	South Marsh Island Block 6	11,200	34.0	0.3	208	Prim.	50.0	45.0	JS	220	100	Succ.	Yes
20	Talco	3,785	23.0	25	147	Prim.	50.0	45.0	Term.	462		Disc.	No
21	Thompson	5,100	25.2	2.7	120	Prim.	42-65	Variable	JS	93	3	Prom.	Yes
22	West Columbia	2,600	30.0	8	116	Prim.	36-48	10-20	JS	290	9	Prom.	Yes
23	West Delta Block 109	10,000	37.3	0.26	194	Prim.	50.0	45.0	JS	3,000	10	Disc.	Yes
24	West Delta Block 109	10,500	36.5	0.28	200	Prim.	50.0	45.0	JS	2,200	300	Succ.	Yes
25	West Delta Block 109	11,325	37.0	0.3	210	Prim.	50.0	45.0	JS	500	10	Succ.	es
26	West Delta Block 109	12,000	34.1	0.25	218	Prim.	50.0	45.0	JS	1,600		Disc.	Yes
27	Wilmington	2,500	14.0	283	123	WF	51.0	30.0	JS	375	375	TETT	TETT
28	Wilmington	2,500	14.0	283	123	WF	51.0	30.0	HF	170	170	Succ.	No
29	Withers North	5,250	25.7	2.45	145	Prim.	35.0	32.0	HF	300	4	Prom.	Yes
30	Withers North	5,320	25.3	2.9	147	WF	32.0	30.0	Term.	98	4	Disc.	No
31	Yates	1100-170	30.0	5.5	82	Gas inj'n			JS	97,341		TETT	TETT

CO_2 FLOODS IN THE UNITED STATES OF AMERICA

Tables 1-M (miscible) and 1-IM (immiscible) list the best known CO_2 floods in the United States. The data in Table 1-M and 1-IM have been drawn from many sources. Most information has come from the 1986 Oil and Gas Journal survey,[3] or from a recent compilation of CO_2 field and laboratory projects.[13] Original literature references to many of the field projects are given in reference 13. Most of the 84 projects in the combined table are producing oil today by CO_2 injection. A few terminated CO_2 projects are still listed because they have been cited so often in the literature that they are useful for comparison purposes.

The locations of the 85 CO_2 floods are given in Figs. 6, 7, and 8. The numbers inside the circles correspond to the sequence number of each project in Tables I-M and I-IM, which are arranged alphabetically by field. Thus, any CO_2-EOR project can be found quickly by field name in the tables and then located on one of the maps. Fig. 6 gives the location for all miscible CO_2 floods except those in the Permian Basin of New Mexico and Texas. These are located more precisely (note that the counties are shown) in the detailed map of that area, Fig. 7. The immiscible floods are located on Fig. 8 which shows that they are concentrated primarily in the Gulf Coast area because of the nature of the reservoirs, or in California because the oil is too heavy for a miscible flood (see following sections). A study of the general map (Fig. 3) along with the actual project locations in Figs. 5-8 shows that CO_2 flooding, except for the immiscible individual well projects, is developing fastest in those areas which have a good natural source of CO_2.

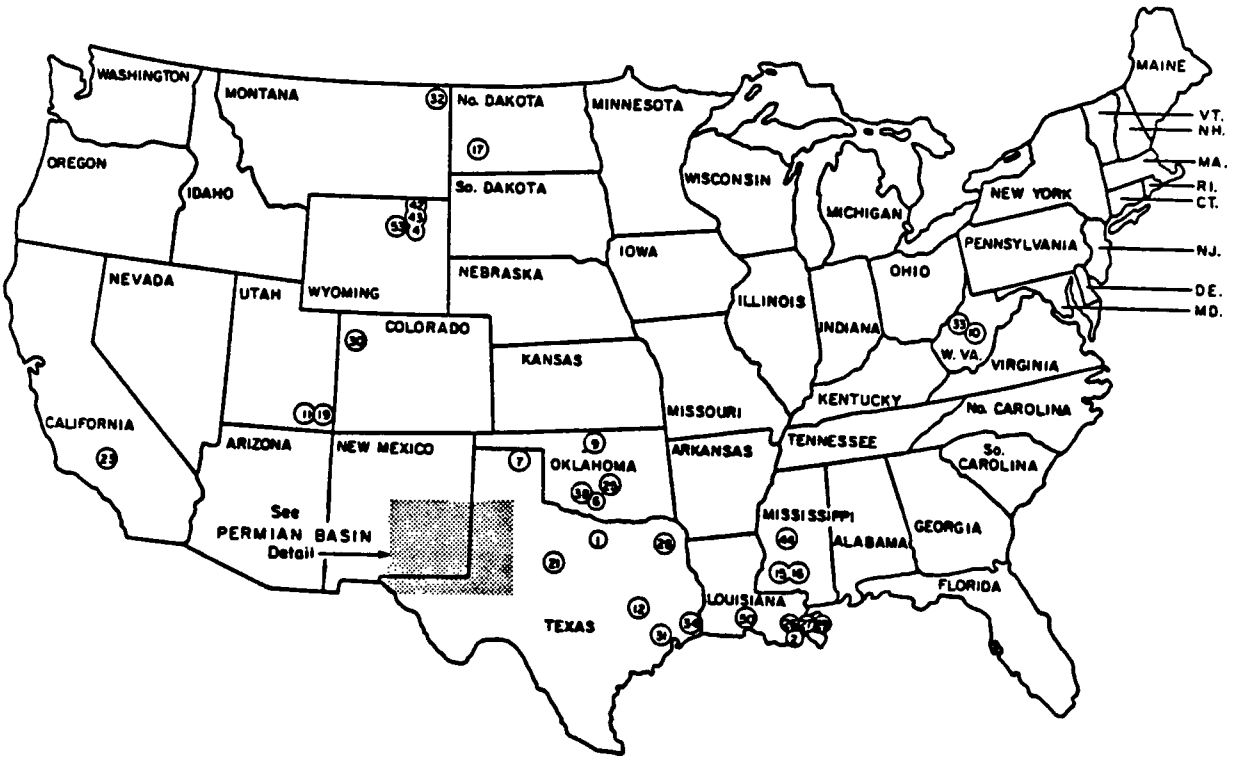

Figure 6. Locations of Miscible CO_2 Floods in the United States.

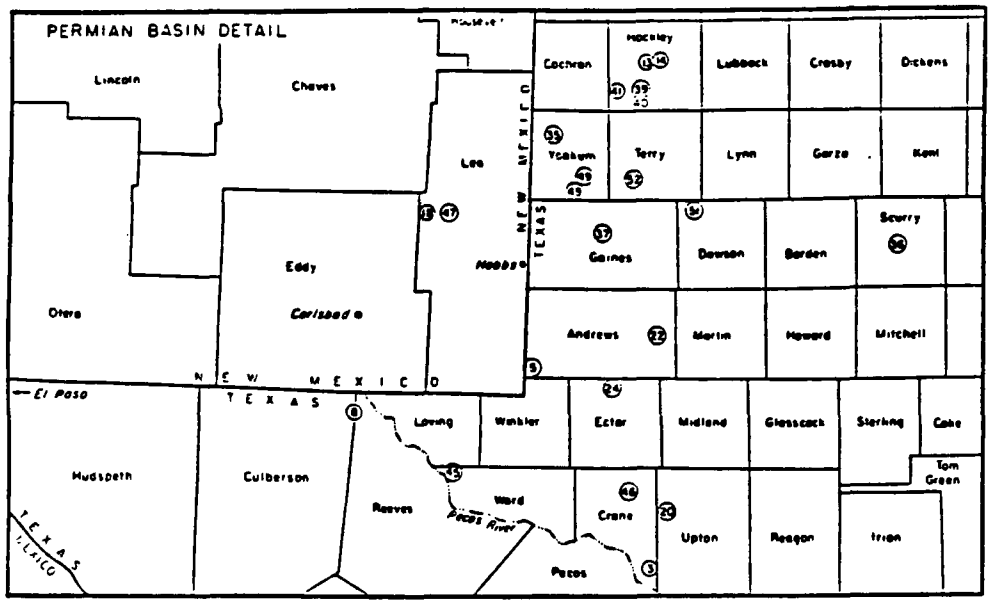

Figure 7. Locations of Miscible CO_2 Floods in the Permian Basin of New Mexico and West Texas.

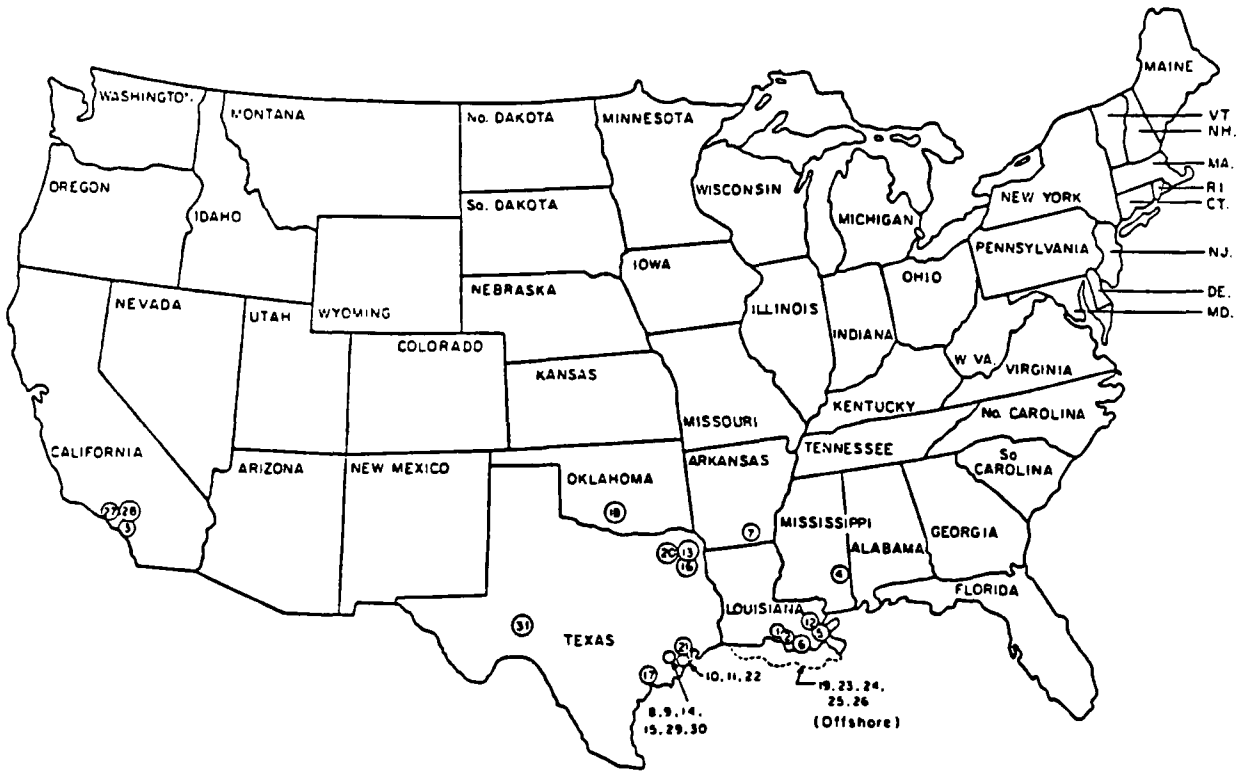

Figure 8. Locations of Immiscible CO_2 Projects.

Miscible vs. Immiscible Projects

The combined Table 1 is divided into 1-M and 1-IM to draw a distinction between the miscible (M) and immiscible (IM) types of projects. The difference between miscible displacement and immiscible displacement by CO_2 or other soluble gases has been considered in several publications.[1,14-16] The usual way to distinguish between the two types of projects is to observe the oil recovery at different pressures in a slim tube test. The general shape of the oil recovery curve by CO_2 in a slim tube displacement is given in Fig. 9. As discussed by many authors, true, first-contact misciblity between CO_2 and common crude oils is never achieved. However, excellent oil recovery is obtained from the idealized porous medium of a slim tube by multiple contact miscibility, as long as the pressure is high enough. This pressure at which excellent recovery is obtained, and beyond which, only insignificant increases occur with added pressure, is called the minimum miscibility pressure or MMP. Normally this occurs at about 95% oil recovery. Obviously, there is a large region (shown in Fig. 9) where oil recovery is significant (and usually much greater than waterflood recovery), but still in the immiscible region. Because of this potential for significant recovery, there has been an increasing interest in immiscible CO_2 floods in the past year, even though the authors of the NPC report did not include immiscible floods in their projections.

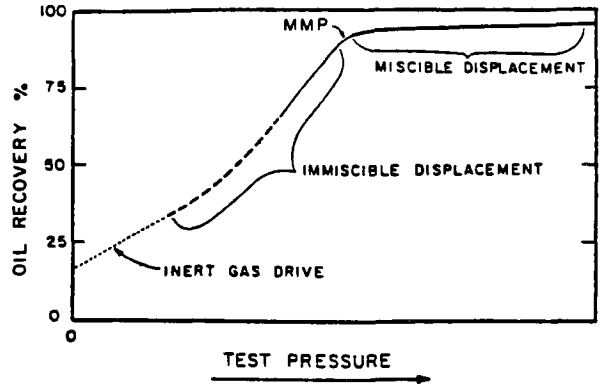

Figure 9. The General Effect of Pressure on Oil Recovery by CO_2 in Slim Tube Tests.

Although data will not be available from most fields, it is instructive to try to assign an oil recovery value which corresponds to a pressure which is a reasonable fraction of the MMP required for the maximum CO_2 recovery. Fig. 10 is an attempt to illustrate this graphically. Some slim tube experiments from the literature[15,17,18] have been replotted in Fig. 10 so that the oil recovery is expressed as a fraction of the pressure required for optimum recovery at the MMP. For most of the curves, the oil recovery at the lower pressures (i.e., at the lower percentages of the MMP) is a long extrapolation. It is assumed that the curves must approach the origin with the general shapes indicated because even a completely immiscible gas drive will give an oil recovery of 15% or more, depending on the oil viscosity. The oil recovery values from three immiscible displacements of the Retlaw (Canada) crude oil are also plotted in Fig. 10. The MMP for the Retlaw

crude oil was not observed, but estimated by extrapolating the data of Sigmund et al.[18]

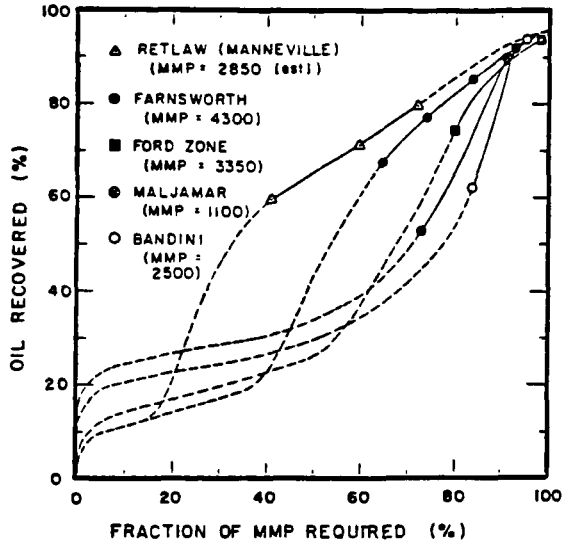

Figure 10. Oil Recovered from Slim-Tube Tests Performed at Various Fractions of the Minimum Miscibility Pressure (MMP). (Calculated from Data in References 15, 17, and 18.)

If one assumes that immiscible CO_2 flooding will be carried out at reservoir pressures which are equal to 75% of the pressures for the MMP, Fig. 10 indicates that the oil recovery should range from 47-83% of the recovery value predicted from the slim tube tests in the laboratory. However, only 95% of the oil is usually recovered in slim tube tests when 100% of the pressure required for MMP is used. Therefore, if oil recovery is compared to the standard NPC model[8] for miscible flooding, the recovery percentages in Fig. 10 should be divided by 0.95. This would mean that the immiscible oil recovery figures should range from 50-87% of the quantities predicted by the miscible NPC model for those immiscible floods carried out at 75% of the pressure required for the maximum recovery at the MMP. Current field results for immiscible projects indicate that recovery should be at least that good, for there is overlap between the oil recovery percentages observed for miscible and immiscible floods. In general, the immiscible oil recovery often appears to be better than predicted by simulation methods which assume that the additional recovery is caused only by oil swelling and viscosity reduction from the dissolved CO_2.[19]

Fig. 11 shows the recoveries which should be expected from the different types of porous media at a range of pressures which spans the immiscible and miscible regions. Note that close to 100% oil recovery can be expected from miscible displacement from a slim tube. However, the oil recoveries drop to only 5-10% at the lower range of immiscible CO_2 floods in the field at those lower pressures.[20] It appears that the slope of the oil recovery versus pressure curve is not as steep for the field projects as it is for the slim tube experiments. Also, as mentioned before, the recovery from some of the immiscible field projects has been better than originally anticipated.

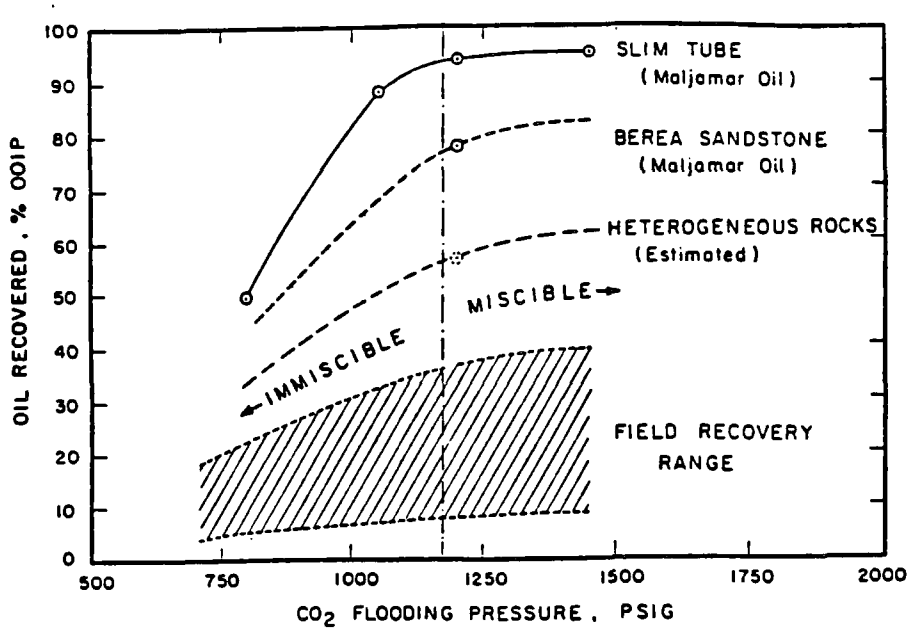

Figure 11. Effect of Pressure on Oil Recovery by CO_2. (After Reference 1 and 20.)

Miscible CO_2 Floods

When most petroleum engineers discuss the major CO_2 field projects in the United States, they are referring to miscible CO_2 displacements, which have the highest potential recovery. It is well known from screening criteria publications that CO_2-miscible field projects are limited by the depth of the formation and the average molecular weight of the crude oil.[7,8,21-23] In general, reservoirs deeper than 2,000 feet, which contain oils lighter than 25° API, are considered candidates for CO_2 flooding. Fig. 12 shows that the pressure required for miscibility (the MMP) increases markedly with the API gravity of the oil, especially at higher temperatures. The MMP required for a given oil increases with depth because the reservoir temperature goes up with depth and the MMP increases with temperature. Fig. 12 gives that relationship between the MMP and temperature for oils ranging between 22-50° API gravity.[24-28] Fortunately, the pressure required to fracture a reservoir also increases with depth because of the heavier overburden. Fig. 13 shows that the pressure available for injection (to avoid parting the reservoir) increases much faster with depth than the pressure that is required for the MMP at the greater depths.[28] Note that the fracture pressure and the MMP, for the 40° API oil shown, intersect at just under 2,000 feet. Therefore, miscible CO_2 projects are rarely found at depths shallower than 2,000 feet, and Table 1-M lists only one such project, Garber at 1,900 feet. The rest of them are distributed at various deeper depths. Most of the miscible projects are arranged by increasing depth in Table 2 which shows that the projects range from the aforementioned 1,900 feet, to the Weeks Island project with a depth of 12,760 feet. It is clear that all of the miscible projects lie within the "window" of Fig. 13, which is an easy way to make a quick screen of a formation if only the depth and API gravity of the crude oil are known. The gravity-temperature relationship for other oils can be cross-plotted easily from Fig. 12 to Fig. 13 as needed.

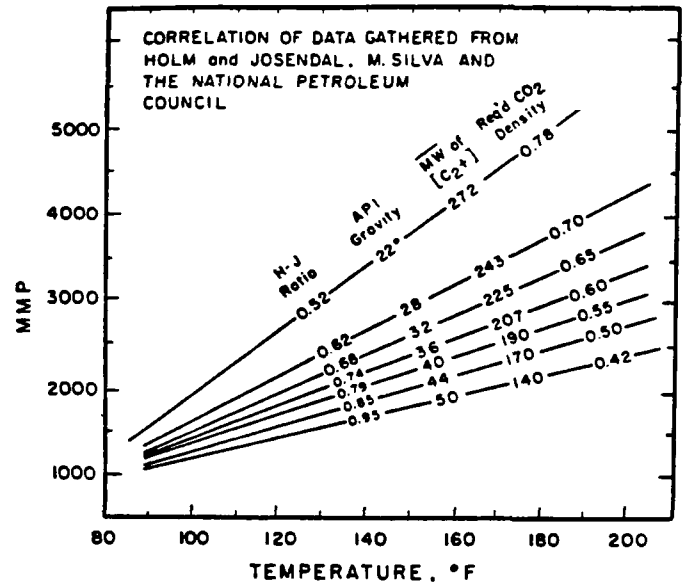

Figure 12. Variation of Minimum Miscibility Pressure with Temperature and Oil Composition (from Data and Correlations of Holm and Josendal,[15] the National Petroleum Council,[8] and M.K. Silva.[24-27]) (After Reference 28.)

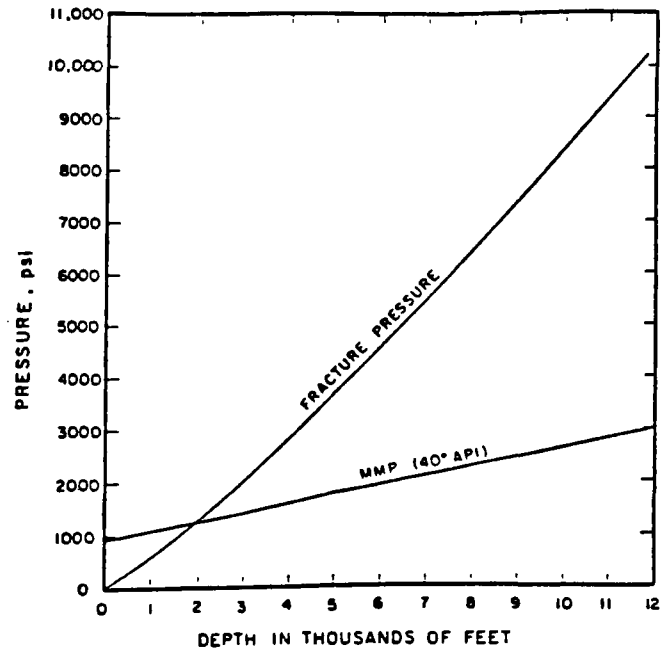

Figure 13. Increase in Minimum Miscibility Pressure (MMP) and Fracture Pressure with Depth for Permian Basin Reservoirs. (After Reference 28.)

Table 2. CO_2 Miscible Projects Arranged by Increasing Depth

Field	State	Depth ft.	----Reservoir oil----- API Gravity	cp	@°F
Garber	Okla.	1,900	44.0	1	100
Ford Geraldine Unit	Tex.	2,680	40.0	1.4	83
West Sussex Unit	Wyo.	3,040	38.0	1.7	93
Maljamar MCA unit	N.M.	3,665	36.0	0.8	90
McElroy	Tex.	3,850	31.0	2.3	86
Means (San Andres)	Tex.	4,300	29.0	6	97
North Cowden Unit	Tex.	4,300	34.0	1.67	94
Vacuum	N.M.	4,500	38.0	1	101
Tinsley	Miss.	4,800	39.0	1.5	175
Welch	Tex.	4,890	34.0	2.15	96
Levelland	Tex.	4,900	30.0	2.3	105
Twofreds	Tex.	4,900	36.0	1.5	105
Slaughter (Estate)	Tex.	4,950	27.0	2	105
Slaughter (Frazier)	Tex.	4,950	31.0	1.4	105
Slaughter (Cent.Mallet)	Tex.	4,950	31.0	1.4	105
Wasson (ODC Unit)	Tex.	5,100	32.0	1.3	110
Sable	Tex.	5,200	32.0	1.46	107
Wasson (Denver)	Tex.	5,200	33.0	1.3	105
Crossett	Tex.	5,300	44.0	0.36	106
Seminole	Tex.	5,300	35.0	1.70	105
McElmo Creek Unit	Utah	5,600	41.0	0.5	125
Alvord South Field	Tex.	5,700	44.0	0.39	154
Greater Aneth	Utah	5,750	42.0	0.47	135
Rangely	Col.	6,000	34.0		160
Sho-Vel-Tum	Okla.	6,200	25.0	3.3	115
SACROC Unit	Tex.	6,700	41.0	0.35	130
Bay St. Elaine	La.	7,400	36.0	0.667	170
Rankin	Tex.	7,900	37.0	0.6	192
Raymond	Mont.	7,900	40.0	0.4	178
Pittsburg	Tex.	8,000	41.0		205
Dollarhide	Tex.	8,000	40.0	0.44	122
Quarantine Bay	La.	8,120	32.0	0.99	183
Rose City North	Tex.	8,200	37.0	2	180
Kurten	Tex.	8,300	38.0	0.4	230
University Waddell	Tex.	8,500	43.0	0.45	140
Paradis	La.	8,600	39.0	0.4	192
North Coles Levee	Calif.	9,000	36.0	0.45	235
South Bishop Ranch	Wyo.	9,200	35.0	1.14	220
Northwest Purdy	Okla.	9,400	38.0	1.2	148
South Bishop Ranch	Wyo.	9,400	34.0		180
Wellman	Tex.	9,800	43.5	0.54	151
Paradis	La.	10,400	37.0	0.5	205
Little Creek Field	Miss.	10,640	38.0	0.4	248
Weeks Island	La.	12,760	32.0	1	225

Table 3 shows the same miscible projects, but this time arranged in order of decreasing API gravity. It is not an accident that the shallowest project (Garber) contains one of the lightest oils in order to fit within the limits of Fig. 13. The projects in Table 3 fall into three almost-equal groups: those fields which have rather light crude oils of 40-44° API, oils of intermediate gravities between 36-39° API, and those ranging from 25-35° API. Therefore, Table 3 shows that almost two-thirds of the miscible CO_2 floods in the United States are carried out in reservoirs which have oils lighter than 35° API.

Immiscible CO_2 Projects

The immiscible CO_2 projects listed in Table 1-IM are immiscible presumably because they are either too shallow or the crude oil is too heavy to meet the MMP criteria for miscible displacement as shown in Figs. 9, 11, 12, and 13. The immiscible CO_2 projects have been arranged in order of decreasing API gravity in Table 4. An examination of this table indicates that some of these projects should meet the criteria for miscible flooding, i.e., their combination of depth and API gravity fall within the window of Fig. 13. The fact that they are reported by the operator as being immiscible floods indicates that sufficient pressures were not available or were not utilized to carry out the normally preferred miscible CO_2 displacement. It must also be emphasized, however, that many of the immiscible floods in Table I-IM are not typical, long-term CO_2 displacements, similar to a waterfloods, but are well-stimulation or cyclic huff 'n' puff techniques (see below). Immiscible projects which are regular CO_2-drive projects seem to be working very well, e.g., the Lick Creek and Wilmington CO_2 floods.[19,29]

Cyclic Or CO_2 Huff 'n' Puff Methods

Traditionally, most enhanced recovery methods involve the injection of a solvent or chemical which drives the oil from the reservoir into a production well. Therefore at least two wells are needed; the large CO_2 floods in the Permian Basin (see Table I-M) often have hundreds of wells; for example, SACROC (No. 36) has 379 injection wells and 887 production wells. These projects utilize repeating injection-production well patterns to develop the large and continuous, near-horizontal reservoirs found in that area. On the other hand, in the highly faulted, saltdome-intruded reservoirs of the Gulf Coast, large horizontal reservoirs are the exception, and repeated, multiwell patterns are not possible in many cases. For these single-well reservoirs, CO_2 can be used to recover oil by the huff 'n' puff method.[30-33]

This CO_2 huff 'n' puff operation is similar to the routine steam stimulation technique used in the heavy oil reservoirs of California. A specific volume of CO_2 is injected into the production well (normally in 1-2 days time), and then the well is shut in to permit the CO_2 to dissolve into the oil. This "soak" period may last for 3-6 weeks, during which time the CO_2 swells the oil and reduces its viscosity. The well is then put back on production. If the treatment is successful, the production rate will be higher than before the CO_2 injection, and it will be sustained for some time. Additional cycles may be performed as long as production increases are observed. Because of the big reduction in viscosity when CO_2 is dissolved in heavy crudes,

Table 3. CO_2 Miscible Projects in Order of Decreasing °API Gravity

Field	State	API Gravity	-----Reservoir oil------- Depth ft.	cp	@°F
Garber	Okla.	44.0	1,900	1	100
Alvord South Field	Tex.	44.0	5,700	0.39	154
Crossett	Tex.	44.0	5,300	0.36	106
Wellman	Tex.	43.5	9,800	0.54	151
University Waddell	Tex.	43.0	8,500	0.45	140
Greater Aneth	Utah	42.0	5,750	0.47	135
Pittsburg	Tex.	41.0	8,000		205
SACROC Unit	Tex.	41.0	6,700	0.35	130
McElmo Creek Unit	Utah	41.0	5,600	0.5	125
Ford Geraldine Unit	Tex.	40.0	2,680	1.4	83
Raymond	Mont.	40.0	7,900	0.4	178
Dollarhide	Tex.	40.0	8,000	0.44	122
Tinsley	Miss.	39.0	4,800	1.5	175
Paradis	La.	39.0	8,600	0.4	192
Kurten	Tex.	38.0	8,300	0.4	230
Northwest Purdy	Okla.	38.0	9,400	1.2	148
West Sussex Unit	Wyo.	38.0	3,040	1.7	93
Vacuum	N.M.	38.0	4,500	1	101
Little Creek Field	Miss.	38.0	10,640	0.4	248
Rose City North	Tex.	37.0	8,200	2	180
Rankin	Tex.	37.0	7,900	0.6	192
Paradis	La.	37.0	10,400	0.5	205
North Coles Levee	Calif.	36.0	9,000	0.45	235
Maljamar MCA unit	N.M.	36.0	3,665	0.8	90
Twofreds	Tex.	36.0	4,900	1.5	105
Bay St. Elaine	La.	36.0	7,400	0.667	170
Seminole	Tex.	35.0	5,300	1.70	105
South Bishop Ranch	Wyo.	35.0	9,200	1.14	220
Rangely	Col.	34.0	6,000		160
North Cowden Unit	Tex.	34.0		1.67	94
Welch	Tex.	34.0	4,890	2.15	96
South Bishop Ranch	Wyo.	34.0	9,400		180
Wasson	Tex.	33.0	5,200	1.3	105
Wasson	Tex.	32.0	5,100	1.3	110
Sable	Tex.	32.0	5,200	1.46	107
Quarantine Bay	La.	32.0	8,120	0.99	183
Weeks Island	La.	32.0	12,760	0.50	225
Slaughter	Tex.	31.0	4,950	1.4	105
Slaughter	Tex.	31.0	4,950	1.4	105
McElroy	Tex.	31.0	3,850	2.3	86
Levelland	Tex.	30.0	4,900	2.3	105
Means (San Andres)	Tex.	29.0	4,300	6	97
Slaughter	Tex.	27.0	4,950	2	105
Sho-Vel-Tum	Okla.	25.0	6,200	3.3	115

Table 4. CO_2 Immiscible Projects in Order of Decreasing °API Gravity

Field	State	API Gravity	Reservoir oil Depth ft.	Reservoir oil cp	Reservoir oil @°F
Paradis	La.	38.0	10,200	0.5	195
West Delta Block 109	La.	37.3	10,000	0.26	194
West Delta Block 109	La.	37.0	11,325	0.3	210
West Delta Block 109	La.	36.5	10,500	0.28	200
West Delta Block 109	La.	34.1	12,000	0.25	218
Lafitte	La.	34.0	8,900	0.7	185
Bayou Sale	La.	34.0	10,000	0.4	194
South Marsh Island Block 6	La.	34.0	11,200	0.3	208
Lake Barre	La.	33.0	13,000	0.4	236
Cote Blanche Bay West	La.	32.0	8,000	1.3	184
Yates	Tex.	30.0	1100-1700	5.5	82
West Columbia	Tex.	30.0	2,600	8	116
Manvel	Tex.	26.7	5,000	7.2	149
Magnet Withers Pierce Estates B&C	Tex.	26.0	5,500	2.3	154
Magnet Withers BH&S State Tracts J.	Tex.	26.0	5,500	2.3	154
Withers North	Tex.	25.7	5,250	2.45	145
Withers North	Tex.	25.3	5,320	2.9	147
Thompson	Tex.	25.2	5,100	2.7	120
Pickett Ridge	Tex.	25.0	4,600	2.5	138
Manvel	Tex.	25.0	4,000	4.4	149
Pierce Ranch	Tex.	24.4	4,900	4.57	155
Sho-Vel-Tum	Okla.	24.0	5,530	18	129
Plymouth	Tex.	23.3	4,650	3.19	150
Talco	Tex.	23.0	3,785	25	147
East Coyote-Hualde Dome Units	Calif	23.0.	3300-4600	12	130
Heidelberg	Miss.	20.0	5,060	15	150
Pewitt Ranch	Tex.	19.0	4,500	30	160
Lick Creek	Ark.	17.0	1100-1700	160	118
Wilmington (1981)	Calif.	14.0.	2,500	283	123
Wilmington (1984)	Calif.	14.0.	2,500	283	123
Pittsburg	Tex.	14.0	3,800	2,200	120

the method was considered as an alternative to the steam huff 'n' puff cycles in the California fields. Recently, encouraging laboratory and field results indicate that the huff 'n' puff method may also provide a good way to utilize CO_2 flooding in the medium gravity oils of the faulted reservoirs of the Gulf Coast.[30,32,33] Monger has shown that the CO_2 requirements and favorable economics may be similar to the results observed in large horizontal reservoirs.[32] Several field examples are described in the references.[30,32,33]

OIL RECOVERY FROM CO_2 FIELD PROJECTS

The largest CO_2 floods in the United States are listed in order of field size in Table 5. CO_2 flooding produces about 70,000 barrels a day of enhanced recovery oil in the United States, and that amount is increasing steadily. Note that the total daily oil production in Table 5 is somewhat larger; in some cases it may be difficult for operators to separate the regular secondary recovery production from the incremental oil which can be assigned exclusively to CO_2 flooding. For this and other reasons, the data on incremental oil from CO_2 injection are missing in the tables in some cases. There are no real surprises in the oil production figures, i.e. most of the expected recoveries fall within the ranges discussed earlier and illustrated in the shaded area for field recoveries in Fig. 11.

Table 5. Twenty Largest CO_2 Miscible Projects in the USA

Arranged by Decreasing Order of Field Size

Field Name	State	Start date	Area, acres	Number Wells Prodn.	Number Wells Inj.	Project maturity	Total prodn. bo/d	Enhanced prodn. bo/d
SACROC Unit	Tex.	1/72	49,900	887	379	NC	43,863	16,000
Wasson (Denver Unit)	Tex.	4/83	20,000	840	280	JS	46,000	1,200
Rangely	Col.	7/86	20,000	360	360	JS	30,000	
Seminole	Tex.	4/83	15,700	328	133	JS	41,800	
McElmo Creek Unit	Utah	2/85	13,440	170	100	JS	5,600	
Greater Aneth	Utah	1982	13,357	140	21			
Northwest Purdy	Okla.	9/82	8,320	106	102	NC	3,500	950
Little Creek	Miss.	12/85	8,200	110	40	JS	3,300	3,300
Wasson (ODC Unit)	Tex.	12/84	7,800	316	250	JS	13,000	
Means (San Andres)	Tex.	11/83	6,700	248	176	JS		
Dollarhide	Tex.	5/85	6,183	62	43	JS	1,900	
Slaughter - (Central Mallet Unit)	Tex.	12/84	5,700			JS	7,500	
Vacuum	N.M.	2/81	4,900	237	97	JS	12,400	80
Twofreds	Tex.	1/74	4,392	42	33	HF	892	892
Ford Geraldine	Tex.	2/81	3,850	198	123	JS	395	395
Welch	Tex.	2/82	2,675	129	132	HF	3,100	300
Alvord South Field	Tex.	1980	2,291	245	10	HF	680	200
Slaughter (Frazier Unit)	Tex.	12/84	1,600	64	37	JS	3,000	
Crossett	Tex.	4/72	1,500	23	11	HF	2,000	2,000
Farnsworth, North	Tex.	6/80	1,472	8	6	HF		
Wellman	Tex.	7/82	1,400	29	2	JS	7,000	

CO_2 Breakthrough Into Production Wells

Experience from almost all of the CO_2 floods in the United States shows that most of the oil recovery occurs after CO_2 breakthrough. Indeed, some of the early floods and pilots may have been stopped too early because the unwanted early breakthrough frightened the operators. Now, until better mobility control is perfected, early breakthrough is accepted calmly as an inescapable part of CO_2 flooding. Fig. 14, which shows the oil and CO_2 production from a single well of the recent West Sussex Unit pilot in Wyoming, illustrates this normal, early CO_2 breakthrough and shows that the flush oil production comes along with produced CO_2 shortly thereafter.[34] Fig. 14 shows that CO_2 breakthrough occurred only one month from the initiation of CO_2 injection. It also shows that the oil production continued to increase for a few months after the CO_2 injection was terminated. In this case, the CO_2-continuous slug was approximately 30% of the reservoir pore volume. It seems clear that the oil which had been mobilized by the CO_2 was moved to the producing well by the waterflood which commenced immediately upon completion of the CO_2 injection. Fig. 15 shows the cumulative CO_2 produced and oil production for the whole pilot. Again, it showed that the good oil kick came after CO_2 breakthrough, and the increase in oil production continued for a long period after the CO_2 injection was completed.

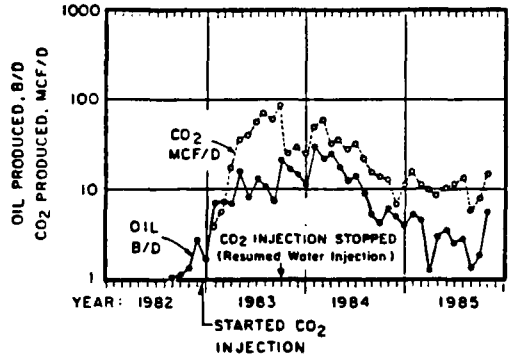

Figure 14. Oil and CO_2 Production for Pilot Well No. 19 of West Sussex Unit. (Project No. 53 of Table 1-M, after Reference 34.)

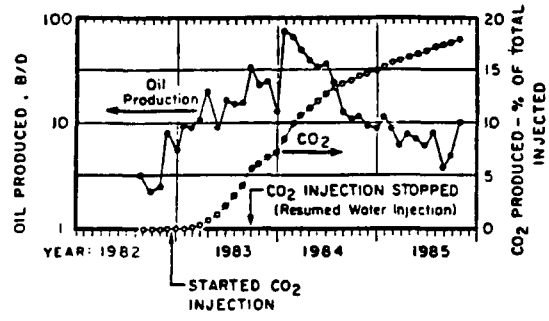

Figure 15. Oil and CO_2 Production from Entire West Sussex Unit Pilot (Project No. 53 of Table 1-M, after Reference 34.)

Continuous vs. Water Alternating with CO_2 Injection

In the West Sussex pilot, the CO_2 was injected continuously until the requisite amount had been pumped into the reservoir, i.e., water alternating with gas (WAG) for mobility control was not practiced, and the operator did not consider CO_2 breakthrough a problem. The controversy over whether to WAG or not to WAG will continue until many more reservoirs have been flooded by both methods. Table 6 will show that the three projects with the highest net oil recovery (Weeks Island, Crossett, and Little Creek) are also projects which have not used the WAG method for mobility control. However, some engineers will point out quickly that this is not a fair comparison. The Weeks Island flood is a gravity-stabilized displacement down-dip, and it would therefore be expected to yield much higher recovery than horizontal floods. The Little Creek early pilot, from which a record 46% of the residual oil was recovered from a watered-out reservoir, used a very large quantity of CO_2, 160% of the hydrocarbon pore volume (HCPV) compared to the common 30-40% HCPV for most field projects. Therefore, it also holds the record for the highest CO_2 requirement: 26 MCF of CO_2 injected per barrel of incremental oil produced. The Crossett CO_2 flood appears to be on the way to very high ultimate recovery, no doubt because it is an enhanced secondary flood in a formation which was too tight for prior waterflooding. Therefore, the oil saturation in the reservoir was very high (compared to tertiary CO_2 floods) when CO_2 injection started. In addition, Crossett was not plagued with early CO_2 breakthrough, perhaps because of asphaltene precipitation and/or multiphase flow which provided added mobility control.[35]

Oil Recovery Observed and Predicted

The oil recovery and CO_2 requirements for several CO_2 floods are listed in Table 6. Note that oil recoveries may range from 10-60% of the remaining oil in place. As mentioned earlier, the enhanced secondary floods show up very well, especially Crossett. This is not surprising because CO_2 flooding is basically a vaporizing gas drive method; if this method is used in a non-waterflooded reservoir, very high oil recoveries can be expected. For example, recovery of 60% of original oil in place is expected from University Block 31 which started with methane injection, switched to flue gas, and finally to nitrogen flooding.[36-41]

As more experience is gained in CO_2 flooding, the general optimism appears to be increasing. For example, published predictions by the same author[29,42] of oil recovery from simulations and other engineering calculations have changed from 1983 to 1986 as follows:

	Expected Oil Recovery (% ROIP)	
	Predicted in 1983[42]	Predicted in 1986[29]
Kelly Snyder (SACROC)	11	21
Crossett	25	55
Twofreds	27	16
Lick Creek	17	24

Table 6. Oil Recovery and CO_2 Requirements for CO_2 Floods

	Observed or Expected Oil Recovery		CO_2-Oil Ratios (MCF/BO)		
	(%OOIP)	(%ROIP)	Purchased	Recycled	Total
• SECONDARY					
Kelly Snyder (SACROC)	10	21	5	2	7
Crossett	44	55	3	7	10
Twofreds	13	16	8	4	12
• TERTIARY					
N.E. Purdy	8	12	4-5	2	6-7
East Velma	9	15	4-5	2	6-7
Little Creek Pilot	18	46	12	14	26
• GRAVITY STABLE					
Paradis	8	10	-	-	-
Weeks Island	-	60	-	-	11-12
• IMMISCIBLE					
Lick Creek	16	24	6	4-5	10-11
General Expected Range (Tertiary)	8-15	15-30	4-8	3-7	7-14

(After Reference 27 and other sources.)

Note that three of the four estimates went up markedly during the past three years. (I do not know why the Twofreds went down.) Indeed, engineers have almost doubled their oil recovery estimate for SACROC and Crossett since 1983. Although "hard copy" references are not readily available, company personnel continue to suggest that the current production figures from the major CO_2 floods often are better than their prior engineering predictions. Seminole, Garber, Slaughter, and Wasson are mentioned as projects which continue to look better and better as more oil production history is accumulated.

It seems clear that the long-term prediction in the NPC report of more than five billion barrels of additional oil by miscible flooding (for which CO_2 will be the major contributor) should be met. A recent article, which includes an update of the miscible flooding results, points out that the present production rate of about 70,000 B/D indicates that the NPC prediction is right on target.[43]

ECONOMICS

The optimism which has been growing because of the good field response from CO_2 injection has been tempered in recent months by the precipitous drop in oil prices. Estimated costs for CO_2 are shown in Table 7 for five of the CO_2 floods that were listed in Table 6. It is presumed that this table

was prepared before the prices had dropped to their lowest values. Table 7 shows that CO_2 costs range from about $4.50 per barrel to less than $9.00 per barrel of incremental oil recovered by the injected CO_2. Therefore the table indicates that it would not be possible to continue floods economically if the price of oil drops much below $10.00 a barrel for those CO_2 floods which consume the most CO_2. However, most of the long-term purchase contracts for CO_2 contain a clause which allows the CO_2 price to drop with a decline in oil prices. Therefore, some of the costs in Table 7 can go down when oil prices are lower. Indeed, Marvin Katz has been quoted as saying that CO_2 can be continued economically with oil prices ranging from as low as $3.00 to $12.00 per barrel as long as the pipelines and the distribution and wellhead equipment are already in place in the oilfields.[44] The oil companies have invested more than two billion dollars in the pipeline supply system for the Permian Basin alone, and as long as any profit margin can be maintained from the produced oil, it is presumed that the CO_2 will keep flowing through the pipelines and into the injection wells.

Table 7. Sample CO_2 Costs for EOR Projects

Project	Purchased CO_2 ($/BO)	Recycled CO_2 ($/BO)	Total CO_2 Costs ($/BO)
• SECONDARY			
Kelly Snyder	4.90	0.60	5.50
Crossett	3.30	1.30*	4.60
• TERTIARY			
N.E. Purdy/ East Velma	4.40	0.80	5.20
• IMMISCIBLE			
Lick Creek	6.20	1.90	8.10

Assuming:
Purchased CO_2 @ $1.00/Mcf
Recycled CO_2 @ $0.40/Mcf
(@ $0.20/Mcf)*

(CO_2 costs can be lower with lower oil prices.)

(Data from Reference 27.)

The startup of new CO_2 field projects is another story. Clearly, one would not want to build a pipeline to inject CO_2 which will cost from $4.00 to $10.00 per barrel of incremental oil, if that oil is going to be sold for less than $10.00. The exact price at which new CO_2 floods can be initiated will depend upon their proximity to existing pipelines. The estimates for oil prices which are needed to start new floods range from $15.00 to $20.00 or even $30.00 per barrel depending upon how far the field is from existing pipelines and a good natural source of CO_2.

Some of the individual states are considering tax incentives (such as forgiving a portion of the production taxes) in order to encourage the initiation of new CO_2 field projects. If this is done, many legislators assume that the increased economic gain from the higher oil production will far outweigh the loss of income from the direct taxes on the oil produced. The New Mexico Research and Development Institute sponsored a recent study to determine if such tax incentives would increase oil production by CO_2 flooding.[45] The results of this study show that some tax relief should have a very positive impact on oil recovery. The study used the methodology of the NPC report[8] to determine the potential of CO_2 flooding for 97 reservoirs in New Mexico. Fig. 16 shows the increased oil production from CO_2 injection that is predicted for oil prices between $20 and $32 per barrel with the present tax structure. Note that the increased oil production is small unless the oil price rises above $24/BBL. However, if proper tax incentives are provided, such as the forgiving of all production taxes until the project recovers the front end costs (i.e., the break even point or incentive to payback on Fig. 17), substantial new reserves, increased oil production, and other benefits to the State will result with oil prices between $20 and $30/BBL. Fig. 17 shows that the increased oil production by CO_2 flooding with tax "incentives to payback" at $24/BBL almost equals the increased CO_2 production at $28/BBL without the incentives. If oil prices rise to $32/BBL, the report concludes that the incentives are not needed to encourage new CO_2 projects.[45]

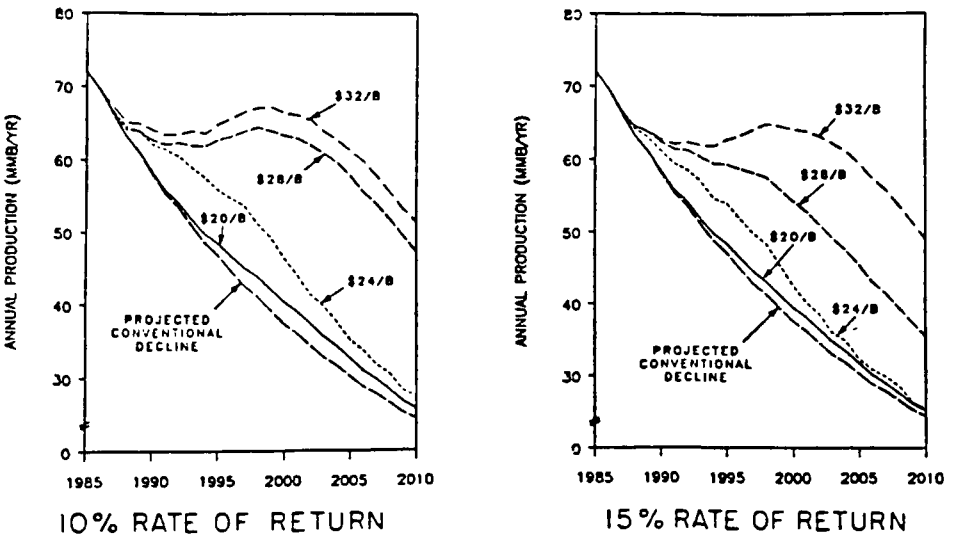

Figure 16. Estimated New Mexico Total Production for Conventional and CO_2 Flooding Techniques as a Function of Oil Price with Current Taxes. (Reproduced from Reference 45.)

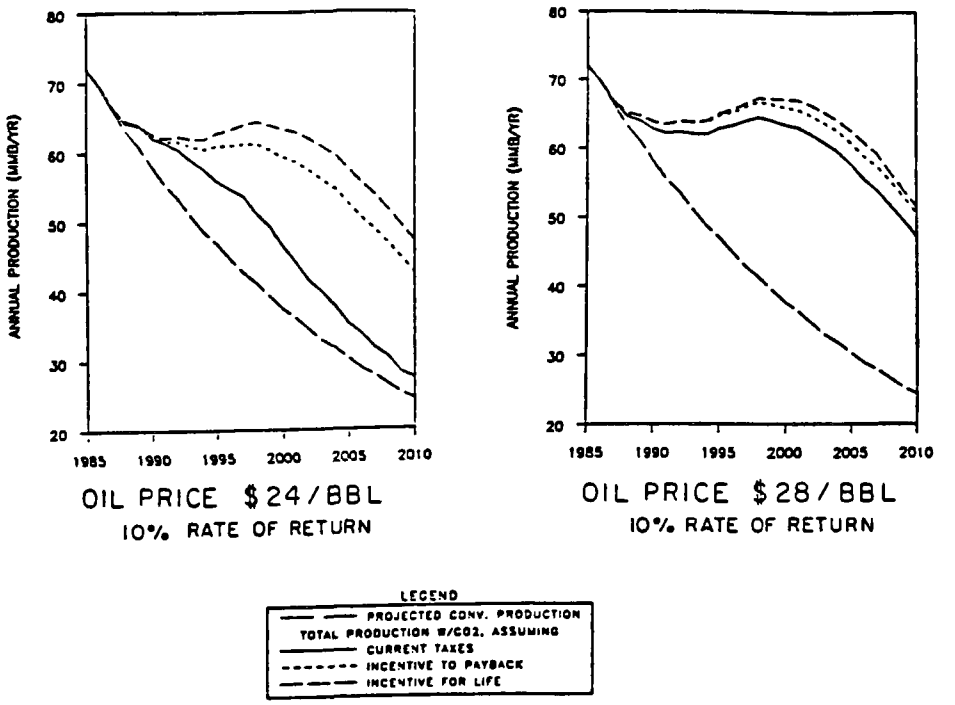

Figure 17. Potential Effects on Total Oil Production Due to CO_2 Flooding in new Mexico for Three Tax Structures at $24 and $28 per Barrel. (Reproduced from Reference 45.)

There have been investigations of the possibility of obtaining CO_2 from power plants in the United States, but with present oil prices, the costs appear to be too high unless a significant "acid rain credit" could be given to some newer processes which are being considered for removing CO_2 from power plant stack gases. Argonne National Laboratories has been investigating the possibility of burning coal in pure oxygen so that the stack gas stream would be almost pure CO_2 plus the acid rain components, all of which could be injected into the ground for good oil recovery. A recent review of past and ongoing flue gas injection projects indicates that the additional SO_2 and NO_x should not be serious problems for the reservoir, and the corrosion problems can be managed[36] (see next section). The quantities of relatively pure CO_2 which could be produced from power plants are very large compared to the volume of the reservoirs which can use CO_2 effectively in the central and eastern part of the United States. If idle pipelines could be reversed to carry low cost CO_2 from the industrial Ohio Valley area to the Gulf Coast oil fields, it should provide a big boost to EOR by CO_2 flooding. Studies indicate that this is feasible.[46]

PRACTICAL FIELD PROBLEMS

There are many more operating problems associated with a CO_2 flood than with a straight waterflood for secondary recovery. Problems which are most often cited are: the early breakthrough of CO_2 plus the continued production of CO_2 throughout the life of the project; the large volumes of CO_2 which

must be separated from the produced oil and gas, and in most cases, recycled; the lower-than-anticipated injection rates which have been experienced in many of the floods; and the hardware problems such as increased corrosion of tubular goods, swelling or deterioration of the elastimers used in gaskets, packers, etc. Although these problems can be serious, all of them seem to be manageable as long as the planning and engineering are done carefully. We have already discussed the fact that early breakthrough is now considered a normal part of CO_2 floods, and the chemical engineers are making great strides in efficient techniques for separating and recycling the produced CO_2.[47,48] Corrosion and problems which may arise from the injection of acid gases into reservoirs are treated briefly in separate sections.

Acid Gas Corrosion Problems

Corrosion has been a problem in oil fields ever since the operators in Pennsylvania started to convert their wooden tanks and pipes to iron and steel equipment many decades ago. Pure oil is not corrosive, but any combination of water with oxygen, or an "acid gas" compound such as SO_2, SO_3, H_2S, NO_x, or CO_2 will normally corrode ordinary carbon steel. Therefore, corrosion engineers expect that CO_2 floods will be more troublesome than waterfloods, but technical problems should have technical solutions, and the corrosion problems with CO_2 are being solved. Corrosion along with other operating considerations are addressed in several references to CO_2 field projects.[49-55]

Flue gases manufactured from methane for injection into reservoirs, and CO_2 obtained from power plants can be much more acidic than the pure CO_2 from the natural sources in the United States. However, flue gas has been injected into oil reservoirs for about 40 years, and the corrosion problems have been dealt with successfully. Table 8 lists seven flue gas projects along with the corrosion control method used.[36] Except for the one started in 1924, all were successful even though corrosion was recognized as a problem.

A well-documented field trial where corrosion was controlled and monitored very carefully was Amoco's Slaughter Estate Pilot which utilized an acid gas composed of 72% CO_2 and 28% H_2S.[54] This mixture was injected successfully for three years with no mishaps except for an occasional shutdown by the automatic safety equipment. Because of the safety concerns, more attention was given to corrosion monitoring and control than in an ordinary CO_2 flood, but the documentation should be very helpful for anyone concerned with acid gas corrosion. The corrosion monitoring system can be summarized by the following list of devices and methods which is reproduced from reference 36:

Corrosimeter® Probes. These devices measured metal loss electrically by the change in resistance across a test probe inserted in the pipe. These probes permitted continuous monitoring of the corrosion rate.

Corrosion Coupons. These mild steel (SAE-1010) rods were inserted into the pipe and removed after specified times to determine metal loss.

Corrosion Test Nipples. Short sections of the same pipe material were cut in half and welded back together with flanges attached so the test sections could be inserted in the CO_2-H_2S flow line at sensitive points, such as at low points in the pipe at injection wellheads.

Ultrasonic Metal-Thickness-Measuring Devices. These instruments were used to measure the piping thickness at various points in the compressor, dehydrator, and gas heater units.

Hydrogen Probes. Corrosion was measured by determining the pressure of H_2 which built up inside a hollow probe inserted into the pipe. The pressure comes from molecular hydrogen (H_2) which formed from the atomic hydrogen ions (H^+) that are formed by corrosion at the probe surface and then diffused through the probe wall. Because the H_2 could not escape, the pressure increased in proportion to the corrosion outside the probe. These hydrogen probes were reported as the most valuable monitoring devices because the H_2 pressures could be read daily and the rate of pressure increase gave a quick indication of change in corrosive environment, including the effectiveness of corrosion inhibitors.

Table 8. Examples of Flue Gas Injection Projects in the United States.

Starting Date	Field Name or Location	Successful?	Corrosion Control	Breakthrough of SO_2 or NO_2 Observed?
1924	Texas	No	Not controlled satisfactorily	No
1949	Elk Basin, Wyoming	Yes	Ammonia injection	No
1959	Louisiana	Yes	Catalytic conversion of NO_x	No
1966 (1949 for methane)	University Block 31, Texas	Yes	Addition of NH_4OH; recycling flue gas around burner tips to reduce NO_x	No
1966	Neale, Louisiana	Yes	Catalytic reduction and excellent dehydration	No
1977	Hawkins, Texas	Yes	Catalytic reduction, corrosion inhibitors, and dehydration	No
1977	East Binger, Oklahoma	Yes	Not reported	No

After Reference 36.

Concerns Related to Interaction of Acid Gases with Reservoir Rock

If Argonne's method for burning coal in oxygen should be adopted by electric utilities, large quantities of CO_2 would become available at low cost. If the stack gas CO_2 is used directly for EOR, questions about the interaction of the reservoir rock and acid rain components in the CO_2 stream will arise. A recent study[36] concludes that an oil reservoir should be an excellent scrubber to remove the acid rain compounds from stack gases. Even though hundreds of billions of cubic feet of flue gas have been injected in oil recovery projects in the United States, Table 8 shows that no NO_x or SO_x has been observed at the production wells. However, most of the flue gas

projects in the United States have injected lower percentages of the acid components because the operators have taken steps to reduce the NO_x concentration to control corrosion prior to the gas injection, and the SO_2 content is very low when sweet methane is used as fuel. Extremely high concentrations of SO_2 might pose a problem in carbonate reservoirs because laboratory flow experiments with 15% SO_2 in CO_2 have gradually plugged limestone cores.[36] However, no plugging, even with pure SO_2, was observed when the gas was flowed through Berea sandstone. Therefore, lab experiments should be carried out before a large project with acidic flue gas is started. Since the SO_2 concentration in the Argonne-process stack gas would be somewhat less than 1% (depending on the coal), it is assumed that many oil reservoirs in the United States could utilize the untreated gas effectively, as long as it was dehydrated enough to control the corrosion.

CONCLUSIONS

CO_2 flooding for enhanced oil recovery in the United States is working, and it works well in either the secondary or the tertiary recovery modes, as miscible or immiscible floods, and with cyclic or continuous CO_2 injection. As more experience is gained from existing floods, the indications are that oil recoveries will be higher than predicted originally. CO_2 from natural sources is available for many of the reservoirs in west Texas and eastern New Mexico, for many other reservoirs in the Rocky Mountain area, and for some reservoirs in Mississippi and Louisiana. Other CO_2 sources will certainly be developed, depending upon the predicted price of oil over the long-term.

The present CO_2 floods will continue to operate with no letup at today's prices (March 1987), but one must wait for indications of higher oil prices before a huge number of new CO_2 floods will be initiated. However, if the present prices hold temporarily, and rise above $20.00 eventually, projections indicate that sufficient CO_2 floods will be underway during the next decade to ensure that the NPC report predictions of 500,000 BBLS/day will be exceeded easily by the time the peak production from CO_2 flooding is reached in the year 2005.

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DESCRIPTION OF A PLANNED CO_2 RECOVERY PROJECT IN WYOMING

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ABSTRACT

Wyodak Resources Development Corp. (Wyodak) has completed studies to determine the market for and the economics of extracting CO_2 gas from the stack gases of a coal-fired electric generating station located in the Powder River Basin near Osage, Wyoming. The results of these studies indicate that there is a market for CO_2 to be used for enhanced oil recovery. This market is, however, dependent on the price of crude oil, the cost to produce CO_2, and the distance of the CO_2 supply from the oil field. Wyodak is prepared to construct a CO_2 plant if contracts can be obtained for its production. If a plant is constructed it will be one of the first plants to be constructed to extract CO_2 gases from a coal-fired electric generating station and could provide a vast new economical source of CO_2 to be used for enhanced oil recovery.

INTRODUCTION

Wyodak is the oldest, continuous operating coal mine in the Powder River Basin. Wyodak is located 5 miles east of Gillette, Wyoming, and has an annual production of approximately 3 million tons of coal per year. Wyodak's parent company, Black Hills Corporation (BHC), is a diversified corporation consisting of an electric utility, a coal mining company, a dry bulk trucking company, and an oil and gas operating company. The electric utility generates, transmits, and distributes electric energy in the Black Hills of South Dakota and a portion of northeastern Wyoming.

BHC has five coal-fired generating stations located within its service area that provide generating capacity to supply its customers' needs. The stack gases of a coal-fired generating station is one of the most plentiful sources of CO_2 gas. The potential users of the CO_2 gas are the oil fields located in the Powder River Basin within a radius of approximately 75 miles of the coal-fired generating stations. The extraction of CO_2 gases from the stack gases of a coal-fired power plant, if done economically, would be a very reliable source of CO_2 for enhanced oil recovery. A CO_2 plant would also be a new source of income for Wyodak and its parent company in utilization of a product that is presently going to waste.

MARKETING STUDY

Wyodak commissioned Stone and Webster Engineering Corporation to do a marketing study to determine the market potential for CO_2 gas in the Powder River Basin area and within a 75-mile radius of BHC power plants. This

study concluded that there was approximately 1.2 billion barrels of oil which could be recovered by the CO_2 enhanced oil recovery method. This recovery would require a demand for 300 million standard cubic feet per day (300MMscfd) of CO_2 gas for the next twenty years. Refer to **Figure 1** for location of potential sources and users of CO_2.

There are two main sources of CO_2 gas--natural deposits of CO_2, and CO_2 available from industrial plant flue gas. The largest single source of industrial plant flue gas is the flue gas from coal-fired electric power plants.

The feasibility of an enhanced oil project is determined by the price of crude oil, the price of the CO_2 available, and the distance of the CO_2 source to the oil field. The marketing study indicated that, for a project to be feasible from BHC power plants in Wyoming, the cost of producing CO_2 would need to be less than $2.00 per Mscf. The feasibility of using CO_2 depends on the price of oil--with each oil field having its own economic conditions of price of CO_2 versus the price of crude oil--to make CO_2 economical.

FEASIBILITY STUDY

Wyodak, in its search to determine if CO_2 could be economically captured from the stack gases of BHC's power plants, hired Pritchard Corporation to do a feasibility study to determine the cost of constructing a plant to extract CO_2 from BHC's Osage coal-fired power plant near Osage, Wyoming. This power plant consists of 3-10 MW stoker-fired units and was picked because it is a base-loaded plant located close to the oil fields. The Osage Plant also has a record of over 90% availability. With three units, an uninterrupted supply of CO_2 could be assured.

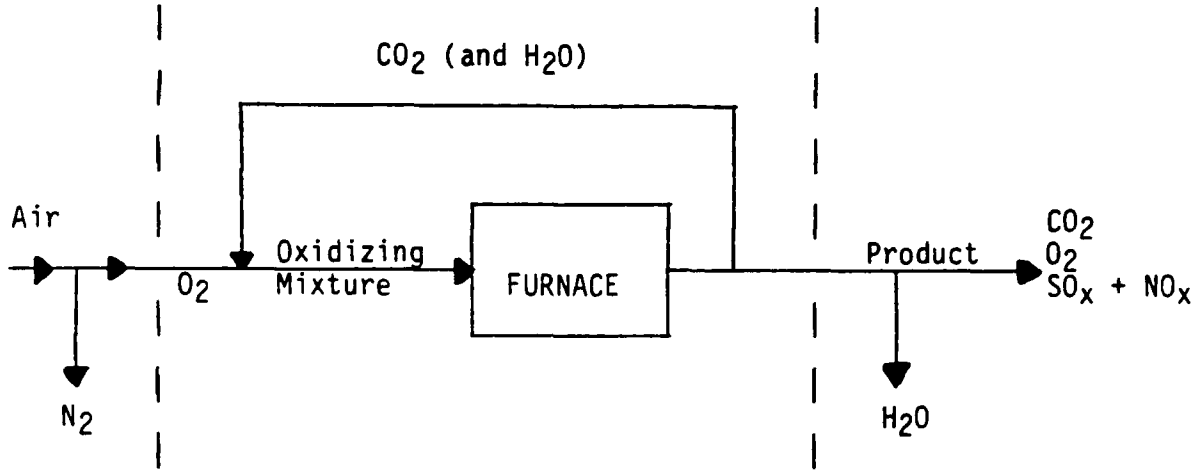
The Pritchard Study surveyed the different methods available to extract CO_2 from stack gases. They then proceeded to calculate the construction and operation costs of a proposed CO_2 plant at Osage. Pritchard's recommendation was to construct a CO_2 plant of approximately 1,000 tons per day CO_2 capacity using the Dow Chemical GAS/SPEC FS-1 solvent and FT-2 technology. This process is based on Dow's proven GAS/SPEC FT-1 technology for removing CO_2 from flue gas plus Dow's new KT-2 (caustic) technology for removing SO_2 from a flue gas. These two processes are combined into their FT-2 process.

The projected sale price of the CO_2 at 2,000 psi delivered at Osage was estimated in the range of $1.50 per Mcft. The total cost of the plant was estimated to be in the $20 million range. These prices are based on 1985 costs and economic conditions. Before a project is started these costs would need to be updated to reflect the new tax laws and cost of capital for construction.

ADDITIONAL STUDY

Wyodak's interest and desire to look at other ways to extract CO_2 from power plant stack gases has led them to work with Alan Wolsky of Argonne

National Laboratories on a new method of obtaining CO_2 from stack gases. This method was originally described by Wolsky at the last CO_2 conference at the Asilomar Conference Center in Pacific Grove, California, on February 11-13, 1985. In this process, a portion of the flue gas flow--which consists of CO_2, H_2O, and other gases--is recycled to the furnace. The recycled flue gas is mixed with pure oxygen to provide firing characteristics similar to air.



Wolsky ran a small test-run using this method at Battelle-Columbus Laboratories. No work had been done on an actual coal-fired boiler. To further Wolsky's work, Wyodak and Black Hills Corporation have recently completed a test using this method on a stoker-fired boiler at BHC's Service Center in Rapid City, South Dakota. The purpose of this test was to determine the feasibility of installing a CO_2 recycle system on commercially sized stoker-fired or pulverized-fired utility boilers.

Summary of Results

The tests showed that it is possible to run the heating boiler in a flue gas recycle mode and to achieve increases in the carbon dioxide levels in the flue gas. Recycle operation did not have noticeably adverse affects on boiler. The equipment for the test consisted of a Keewanee fire-tube boiler with Canton (Detroit Stoker) stokers. It is rated at 2.2 million Btu's and is fired on Wyodak sub-bituminous coal. The coal is a low sulfur coal with approximately 8,000 Btu's per pound. The boiler is equipped with forced draft fan and induced draft fan. The unit normally operates with balanced draft. It provides 15 psi, 230 degree Fahrenheit hot water to the heating system.

Equipment Modifications

The major modification to the system consisted of installing the bypass ductwork to provide the flue gas recirculation capabilities. The bypass duct was 12 inch round, insulated duct. It had provisions for the installation of the sparger near the connection to the flue gas duct. It also had slide gates to allow normal, air-fired conditions

as well as oxygen-flue gas firing. A damper was installed in the flue gas duct after the bypass connection. The damper provided a means of varying the recycle percentage.

Oxygen Equipment

The oxygen supply equipment was provided by Linde Division of Union Carbide Corporation. This equipment included a LOX storage tank, evaporators, safety valves, and flow control equipment. Argonne, Linde, and Black Hills Power personnel reviewed the project from the safety standpoint. All piping was installed according to Linde recommendations. This included pickling the one-inch copper supply lines, silver soldering all fittings, and purging the system with the nitrogen before each operation. Interlocks were installed to shut off the oxygen due to parameter excursions such as high bypass duct temperature, no bypass flow, high excess oxygen, and high stoker temperature.

Boiler Operation

The boiler operation had three phases, normal air fired, transition to oxygen-flue gas, and oxygen-flue gas test. The boiler was started under air fired conditions. The slide gate at the Force Draft Fan was opened to the atmosphere, the slide gate at the sparger was closed and the damper was fully opened. The transition to oxygen enriched was relatively simple because the coals in the fuel bed would "hold the fire" during the transition. Recycle was started by closing the two slide gates and partially closing the damper. The oxygen feed was started and gradually increased to the test level. Forced Draft and Induced Draft fan settings were then adjusted to get the least negative wind box pressure.

Observations of the fuel bed seemed to indicate that the size of the fuel bed was a governing factor on efficient combustion. This was indicated by high level of CO readings when the bed was at a larger than normal level even though excess oxygen was observed in the flue gas. When the bed burned into the stoker slot (grate), excessive grate temperatures were sometimes observed. However, CO levels were very low and flame conditions were very good with a small bed. The operator observed that when firing with the small bed (O_2 enriched) the clinkering appeared to be less than during normal operations. Also, the stack was visually cleaner. The boiler required more operator attention during O_2 enriched firing.

Conclusions of Additional Study

The tests provided some very useful information concerning the possible retrofit of an existing unit to CO_2 recirculation.

1. The transition from air to O_2 enriched firing did not seem to be difficult. It was accomplished several times during the tests without any problems at all.

2. No extraordinary training was required for the operators to feel comfortable operating the boiler under recycle conditions.

3. Gas leakage, both air into the system and flue gas out, will require significant attention with any commercial unit. Apparently, minor leaks can limit the purity of the CO_2 in the recycled gas. Existing stoker-fired boilers that were not designed for leak tight boiler settings would be difficult to retrofit. Pulverized coal boilers, especially those with pressured furnaces, would probably be good candidates for retrofit. These types of boilers are already designed with relatively tight settings. Moderate modifications would be required to obtain a suitable leak-free system. Blanketing the coal bunker with CO_2 will probably improve the CO_2 levels in the recycle gas significantly.

4. Safety considerations in a commercial unit should address the hazards of handling the pure oxygen. Safety interlocks that would prevent explosions in the boiler from high O_2 levels would be one item. Prevention of pure oxygen leaks into the plant would be addressed as in any other industrial facility. The other major safety consideration would concern the fact that Carbon Dioxide and Carbon Monoxide are not life supporting atmospheres. Permanent monitoring and alarms would probably be required to prevent accumulations of high concentrations in the plant. Special precautions would also be required for the entry to enclosed areas such as coal bunkers.

Economical consideration will dictate which method of CO_2 recovery Wyodak would recommend using. The Dow Chemical FT-2 process or the Argonne oxygen process. The timing for construction of a CO_2 plant will ultimately depend on the price of crude oil.

POTENTIAL SOURCES AND USERS OF CO2

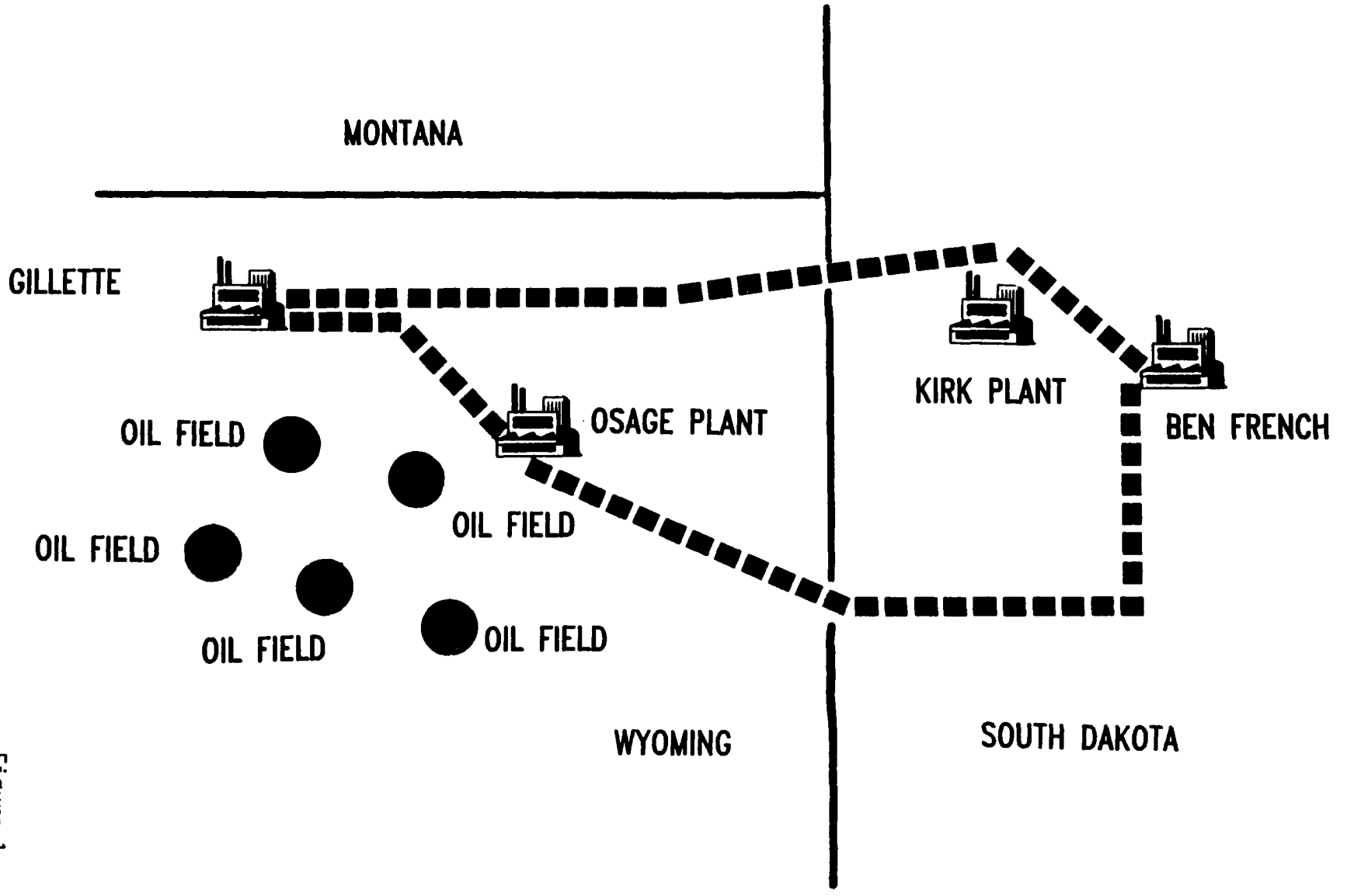


Figure 1

RECOVERING CO_2 FROM STATIONARY COMBUSTORS: A BONUS FOR ENHANCED OIL RECOVERY AND THE ENVIRONMENT

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ABSTRACT

Argonne National Laboratory is conducting research on a new approach to recovering carbon dioxide from stationary combustors. This research is aimed at providing the private sector with the information it needs to decide whether the approach can contribute to future supplies of carbon dioxide for enhanced oil recovery. The approach includes the simultaneous recovery of other gaseous combustion products, such as oxides of sulfur and oxides of nitrogen. The product stream, essentially all carbon dioxide, could be used for enhanced oil recovery. The approach also may find application where strict air pollution controls are mandated.

INTRODUCTION

Enhanced recovery of oil by carbon dioxide flooding is one of the fastest growing oil production methods in use today, with the possibility that 500,000 barrels per day could be produced by this method by the year 2000.[1] According to the National Petroleum Council, oil production by CO_2 miscible flooding will surpass thermal recovery in 20 years.[2] Currently, carbon dioxide sources fall into two broad categories: natural deposits (or plants at which carbon dioxide is already recovered and vented) or plants with carbon dioxide present in dilute vented streams. Argonne National Laboratory has concentrated its research into carbon dioxide recovery on the second category, specifically on recovery from power-plant flue gases.

The conventional approach to post-combustion recovery of carbon dioxide is to separate it from stack gas, which is expensive because carbon dioxide is a relatively small fraction of the stack gas (about 15% by volume), and because the stack gas includes various molecular species that interfere with carbon dioxide recovery. Conventional techniques use monoethanolamine (MEA) or hot potassium carbonate systems, or variations of such systems, to absorb carbon dioxide from stack gas. The absorbing material is then regenerated and the carbon dioxide is driven off and recovered as a gas. Difficulties with the conventional approach include the expense of boiler duty for regeneration, the need to reduce the concentration of oxygen in the flue gas, and the fact that sulfur oxides (SO_x) poison the solvent. The last consideration is very important when considering carbon dioxide recovery from combustion of heavy oil or coal.[3]

The concept being investigated by Argonne permits total recovery of the carbon dioxide and other gaseous combustion products (SO_x and nitrogen oxides, NO_x). In this approach the procedure is as follows:

- Incoming air is separated into O_2 and N_2 streams by a facility adjacent to the combustor. The nitrogen is immediately returned to the atmosphere or, in a fortunate case, sold as a by-product.
- The oxygen stream is mixed with carbon dioxide and other inert gases from a recycled flue-gas stream.
- This mixture (which is 70% CO_2 and 30% O_2 by volume) is used instead of air to burn the fuel.
- After heat exchange is complete, the resulting gas (about 91% CO_2, 7% H_2O, and 2% O_2 by volume, with small quantities of SO_x, NO_x, etc.) is divided into a product stream and a recycled stream.
- Finally, the product and recycled streams are further conditioned as desired (water could be removed, leaving a stream that is 95% CO_2, 3-4% O_2, and 1-2% SO_x and NO_x by volume; carbon dioxide with liquid water may corrode pipelines, while "dry" carbon dioxide will not).[3]

Since this approach involves no gaseous emissions after combustion, a combustor operated this way would need no air-pollution control equipment, either conventional or of an advanced type.

STATUS OF RESEARCH

Argonne has conducted five research projects to prove this concept. These projects, described in detail in Refs. 4-8, are listed and discussed briefly here:

- An Argonne-designed experiment, performed by Battelle Columbus Division, to obtain sufficient experimental data to identify relative differences between coal-air and coal-CO_2-O_2 flames.[4]
- Computer modeling to simulate the heat transfer that results from burning coal in a mixture of CO_2 and O_2 rather than in air.[5]
- Experimental testing using wet recycle, at the 2-million-Btu/h scale.[6]
- Evaluation, with the help of a detailed furnace computer model, of the impact of using CO_2-O_2 or CO_2-H_2-O_2 mixtures as an oxidizer (instead of air) on the thermal performance of a coal-fired boiler.[8]
- Experimental testing using wet and dry recycle, at the 10-million-Btu/h scale.[7]

Evaluation of Firing Pulverized Coal in a CO_2-O_2 Atmosphere[4]

This project compared the performance of a test combustor firing coal in a CO_2-O_2 mixture to performance when firing in air, and it provided a basis for inferences about the effect of this substitution in larger combustors. Specifically, this work:

- Fired coal in air and in CO_2-O_2 at three mixture ratios,
- Measured the important combustion parameters for both coal-air and coal-CO_2-O_2 combustion,
- Determined the mixture of carbon dioxide and oxygen that provides a flame with a total radiant heat flux similar to that of a coal flame burning in air, and
- Estimated the effect of the combustion atmosphere composition on combustion efficiency, emissions, deposits, and other items of interest to boiler designers.

This multiple-test experiment, designed by Argonne, was conducted by Battelle Columbus Division at its combustion facilities. A water-jacketed, refractory-lined cylindrical furnace (2 ft x 7 ft) was used, with tubes simulating a superheater placed downstream. The furnace was fired with pulverized coal (about 400,000 Btu/h). A baseline test used air as the combustion atmosphere; other tests used various mixtures of carbon dioxide and oxygen.

These tests resulted in important evidence of the technical feasibility of the Argonne approach, providing data for comparing coal-CO_2-O_2 firing with coal-air firing. Results indicated that the process, firing coal in a large utility boiler in an atmosphere of recycled flue gas and added oxygen, is technically feasible. This conclusion was based on the similarities between firing coal-air and coal-CO_2-O_2 in regard to combustion characteristics, radiant heat transfer, and emissions.

Model of Furnace Heat Transfer for Combustion in CO_2-O_2 Atmospheres[5]

During the Battelle Columbus test, Argonne developed a one-dimensional model of heat transfer from a cylindrical combustor. The model simulates the heat transfer from fossil-fuel combustion when air or a CO_2-O_2 mixture is used as the oxidant. The coal feed rate, combustor dimensions, and other model parameters are the same as those specified by Argonne and used in the Battelle tests described above.

The Argonne model effectively predicted heat transfer in the coal-air burn and in the three CO_2-O_2 burns. These findings lend further credence to the experimental results cited above and to the general feasibility of burning coal in CO_2-O_2 rather than air.

The small-scale tests conducted by Battelle suggest that combustion of coal with mixtures having CO_2-to-O_2 molar ratios between 2.23 and 2.42 yields heat transfer and combustion characteristics similar to those seen in air. These tests were conducted using mixtures of pure CO_2 and O_2 to look at the feasibility of the fundamental process. The next steps in the development process were to consider some of the practical aspects of the process, including:

- Evaluating, at a realistic scale, the practical feasibility of converting a furnace system from coal-air combustion to coal combustion in a mixture of oxygen and recycled flue gases.

- Identifying the ratio of recycle gas to input oxygen that is needed to achieve heat transfer performance similar to that of coal-air combustion, and quantifying any changes in important parameters (such as burnout and flame stability) that might affect overall system performance.

- Providing a basis for scaling experimental results up to larger commercial, utility-scale equipment.

The Argonne approach raised crucial questions for research: Will fuel (particularly coal) burn normally in mixtures of carbon dioxide and oxygen or in mixtures of carbon dioxide, oxygen, and water? If it will, will normal heat transfer take place with such a burn? What practical problems will be encountered when retrofitting Argonne's new method to an existing furnace being operated by its usual staff? To answer these concerns, Argonne directed a project with the Black Hills Power and Light Company in Rapid City, South Dakota.

Tests to Recover CO_2 at the Black Hills Power and Light Company[6]

A 2.2-million-Btu/h, coal-fired, stoker-fed boiler was retrofitted for wet-recycle CO_2 recovery by the staff of Black Hills Corp., the owners and operators of the furnace. Two related modifications -- sealing the brickwork supporting the boiler and blanketing the coal bunker with carbon dioxide -- were beyond the scope of this retrofit and test, although they would be necessary for practical operation of a stoker furnace retrofitted for recovery of carbon dioxide. Linde Division of Union Carbide provided oxygen and the associated plumbing, and Argonne provided instrumentation and staff to monitor the tests.

The modified utility boiler was instrumented to examine the feasibility of producing and recovering carbon dioxide by burning coal in oxygen and recycled flue gas in a utility environment. The tests demonstrated that the boiler can be operated in the oxygen-blown/flue-gas-recirculation mode without any noticeable effects on coal combustion, heat delivery to the water, or the coal-feed or ash handling systems.

Pretest calculations showed that a feasible set of operating parameters for a CO_2-producing combustor system (tightly sealed against air infiltration and containing no

more than about 5% O_2 [dry basis] at the furnace exit) would be a flue-gas recycling ratio between 0.6 and 0.7 and an oxygen feed rate of 1.17 g-moles per g-atom of carbon, yielding an exhaust gas composition (wet basis) of approximately 46.9% CO_2, 50.6% H_2O, and 2.5% O_2 (dry basis). However, because air leaked into the test combustor and the flue-gas handling system, the highest carbon dioxide concentration achieved in the exhaust gas was 48.5% (dry basis). Major sources of in-leakage were the furnace brickwork, the gas-handling system, and the coal-feed and ash-extraction systems.

Two-Dimensional Modeling of Fossil-Fueled Power Plant Behavior[8]

A comprehensive analytical study investigated how the thermal performance of a utility boiler is affected when air is replaced by mixtures as the oxidizer. The study was performed using an Energy and Environmental Research Corporation heat transfer and combustion zone model that incorporates state-of-the-art methods for predicting the performance of fossil-fuel-fired boiler furnaces.

The model is based on local heat and mass balances solved for various arrangements of furnace zones. Radiative heat exchange between all furnace zones, which is the dominant mode of heat transfer in the radiant section of a boiler, is accurately simulated by use of Monte Carlo calculation techniques. The model requires specification of certain input data, including a description of the furnace flow distribution and the distribution of wall deposits, which are considered to be the key parameters. The boiler selected for the performance study was a tangentially fired coal combustor.

The study indicated that optimal CO_2-O_2 or CO_2-O_2-H_2O molar ratios exist at which a particular boiler can be operated with these mixtures in a way that performance changes are minimal compared to the air operation for which the boiler was designed. These ratios were later found to be compatible with the experimental results cited below. The main criterion for determination of the optimal molar ratios was achievement of heat transfer efficiencies (for the dry- or wet-recycle process) that are, at full load, the same as for air operation.

Pilot Tests to Simulate a Typical Utility Boiler Fired with Pulverized Coal[7]

While tests were underway at the Black Hills plant, pilot-scale experiments were being conducted by the Energy and Environmental Research Corporation at its Tower Furnace facility. The tests were conducted at a scale of 10 million Btu/h with the facility configured to simulate both the geometry and thermal environment (temperature-time history) of a typical utility boiler fired with pulverized coal. The Tower Furnace, which is fired by a single, variable-swirl coal burner, has multiple access ports for sampling and observation, incorporates many features characteristic of full-scale boilers (such as a simulated superheater section, a tubular air heater, and fly ash removal), and is equipped with a full complement of measurement and control instrumentation.

The base program was conducted with a series of trials to establish the optimal flue gas and oxygen mixture that would produce performance matching conventional combustion

in air. The baseline air condition was characterized with a subbituminous coal from Wyoming. A range of recycle gas/oxygen mixtures were tested and the resulting performance compared with the baseline air case. During this basic test program, the flue gas was wet -- that is, the water vapor from combustion was not removed. A comprehensive series of measurements were made to quantify (1) carbon burnout, (2) flame stability, (3) heat transfer performance in both the radiant and convective furnace section, and (4) slagging, fouling, and ash deposition throughout the system.

In addition to this base program, a series of optional tests were conducted to more fully characterize this recycle gas/oxygen combustion process and extend the evaluation to a wider range of operating conditions. These additional tests included:

- Evaluation of a highly volatile, bituminous western coal,
- Evaluation of reduced load operation with the recycle gas/oxygen combustion process,
- Modification of the furnace system to accommodate evaluation of dry recycle gas,
- Detailed in-furnace measurements to fully characterize selected conditions, and
- Two-dimensional heat transfer modeling of the furnace performance to provide a tool for extrapolation and evaluation of data, as well as a link to other experimental and theoretical studies.

This program demonstrated that pulverized coal can be burned satisfactorily in mixtures of pure oxygen and recycled flue gases, under conditions representative of utility boilers. Optimal flue gas recycle ratios were found for which performance changes were minimal compared to operation on air. For the wet-recycle process, where flue gases are recycled without drying, the optimal recycle ratio was found to be about 3.25. For the dry-recycle system, where a substantial fraction of the flue gas moisture had been removed, the corresponding optimal recycle ratio was found to be 2.6.

For both recycle conditions, measurements showed the heat transfer to decrease with increasing recycle ratio, with heat transfer to the cooled water-wall panels showing a slighter stronger dependency on the ratio than did heat transfer to the hot refractory walls. Although the scale of the experimental system is still small (10 million Btu/h) compared to full-scale utility boilers, care was taken to simulate overall heat transfer characteristics, and the heat transfer results are believed to be a favorable indication of the potential for full-scale application.

Other performance parameters -- such as flame stability, carbon burnout, and slagging and fouling tendencies -- were found to undergo minimal changes for optimal recycle conditions, compared to baseline operation in air. However, NO_x and SO_x emissions were found to be quite sensitive to the recycle process. Surprisingly, the emissions of NO_x were reduced by about 70% under optimal dry-recycle conditions and by about 80% for

wet recycle, compared to baseline values recorded for air. Emissions also decreased in direct proportion to the recycle ratio applied. This behavior is believed to be due to incineration (or reburning) of recycled NO_x as it passes through the main combustion zone, and it is a characteristic expected to recur in full-scale applications.

Emissions of SO_x also were substantially reduced under recycle conditions. For wet-recycle operation, this result is believed to stem from a relatively inefficient particulate removal device, which allows fly ash to be recycled through the furnace, providing an opportunity for enhanced use of the inherent alkali material. Under dry-recycle operation, the water removal device acts like a wet scrubber, also increasing the use of alkali material in the fly ash.

In operating the furnace system, few problems were experienced. It was necessary to prevent air ingress into the system that would reduce the purity of the carbon dioxide product. In addition, it was necessary to install additional fan capacity to handle the required volume of hot, recycled flue gases. Air in-leakage limited the carbon dioxide concentration in the flue gas to 94% in the test furnace, an acceptable concentration.

CONCLUSION

Overall, the results of the studies described above, along with results of the other work Argonne has undertaken concerning the recovery of carbon dioxide from stationary combustors, indicates that the process may be applied successfully as a retrofit to a wide range of utility boiler and furnace systems.

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CONFERENCE AGENDA

March 19, 1987

9:00 a.m.	Introduction and Welcome *Alan Wolsky, Argonne National Laboratory*
9:15 a.m.	Current Status of the Use of CO_2 for Enhanced Oil Recovery *Joseph Taber, New Mexico Petroleum Research Center*
10:00 a.m.	Break
10:15 a.m.	A New Method for Recovering CO_2 from Stationary Combustors *Alan Wolsky, Argonne National Laboratory*
11:00 a.m.	Description of a Planned CO_2 Recovery Project in Wyoming *Dick Tupper, Wyodak Resources*
11:45 a.m.	Lunch
1:00 p.m.	Description of a North Sea Enhanced Oil Recovery Project *Ray Park, Oil and Petrochemical Consultant (U.K.)*
1:45 p.m.	Potential CO_2 Sources, Costs and Risks *William B. Johnson Jr., Big Three Industries*
2:30 p.m.	Break
2:45 p.m.	Potential Need for Man-Made CO_2 in Enhanced Oil Recovery *Tom Shepard, Production Operators, Inc.*
3:30 p.m.	Discussion Session
4:00 p.m.	Adjournment

March 20, 1987

9:00 a.m.	Introduction *Alan Wolsky, Argonne National Laboratory*
9:15 a.m.	Environmental Issues of Coal Combustion *Charles Hakkarinen, Electric Power Research Institute*
10:00 a.m.	A Perspective on the Greenhouse Effect and CO_2 Flue Gas Recovery for EOR *Ralph Rotty, University of New Orleans*
10:45 a.m.	Break
11:00 a.m.	Description of the Test Results of the Argonne Coal Oxygen Process *Roy Payne, Energy and Environmental Research Corp.*
11:45 a.m.	Discussion Session, Lunch, and Concluding Remarks *Alan Wolsky, Argonne National Laboratory*
1:30 p.m.	Adjournment

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