UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

May 6, 2006

SUBJECT: Comprhensive Data Evaluation Record (DER) of three separate volumes:

"Determination of Dislodgeable Carboquat Residues from Sapwood Boards Pressure Treated with an Ammoniacal Copper Quat (ACQ) Formulation" (MRID 467640-01)

"Comparison of Dislodgeable Residue from Carboquat Pressure Treated Lumber Using Hand Rubbing versus Rubbing with Wet and Dry Polyester Wipes" (MRID 467640-02)

"Validation of Methods to Extract and Analyze Carboquat Residues from the Palm of the Human Hand and from Dry and Wet Polyester Wipes" (MRID 467640-03)

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DP Barcode: 327357

The attached document contains a separate Data Evaluation Report for each of the three MRID numbers submitted by Lonza Inc. under this barcode.

DATA EVALUATION RECORD: "Determination of Dislodgeable Carboquat Residues from Sapwood Boards Pressure Treated with an Ammoniacal Copper Quat (ACQ) Formulation" (MRID 467640-01)

EXECUTIVE SUMMARY:

The purpose of this study was to determine the amount of carboquat that will be dislodged from pressure treated wood under conditions that represent consumer exposure as a result of treatment with Reg. #6836-236, which contains 50% a.i.. Transferable carboquat residues were collected from sapwood Southern Yellow Pine (SYP) boards that were pressure treated with an ammoniacal copper quat (ACQ) formulation at a commercial wood treatment facility. The ACQ formulation was prepared using the manufacturing end use product Q50-C, which contained approximately 52% carboquat. The resulting ACQ formulation contained between 0.29 and 0.34% carboquat. The boards were treated to a nominal preservative retention rate of 0.25 pcf (pounds per cubic foot) following standard commercial practices. Eight days after treatment, the boards were placed in an outdoor environment in Ontario, Canada where they were exposed to natural sunlight, rain and other natural conditions. Wipe samples were collected on 8, 14, 21, 28, and 35 days after treatment (DAT) using a dry polyester wipe technique, and none of the areas were sampled more than once. At each sampling interval, three replicate samples were collected from each of three treatment batches (total of 9 samples per sampling interval) and one control sample was collected from each of the three batches of untreated wood.

To determine if there were any significant differences in the dislodgeable carboquat residues between the three treatment batches for a specific sampling day, the registrant did provide the results of a multiple t-test (two tail) comparison. The results of this indicated that there were no statistical differences between the treatment batches, and the treatment batch replicates were able to be combined for all further analyses. Dislodgeable carboquat residues were collected using the dry polyester wipes and the amounts were provided in the raw data as obtained in micrograms. These values were corrected for percent recovery, and subsequently, divided by the area of the sampled wood surface (500 cm^2). These calculations provided by the registrant allow for a presentation of and conclusions to be drawn about the amount of dislodged carboquat from treated SYP.

The dry polyester wipe residue data were transformed to be representative of theoretical residues that would transfer from the pressure treated wood boards to wet hands. The data were transformed by dividing the carboquat residue collected from the dry polyester wipes by the transfer reduction factor calculated from the study "Comparison of Dislodgeable Residues from Carboquat Pressure Treated Lumber using Hand Rubbing Versus Rubbing with Dry Polyester Wipes" (MRID 467640-02). A separate discussion of this MRID is provided later in this reprot. The transfer reduction factor is the ratio of carboquat residues dislodged by wet hand rubbing relative to that dislodged by dry polyester wipes. The Agency is most interested in what the dislodgeable residues are for wet hands because it is the most representative of the worst-case scenario for human contact with the treated boards (e.g. child playing on a deck). After transforming the data for wet hands, the average dislodgeable residue and standard deviation for all of the treatment groups combined are summarized in Table 1:

Table 1: Amount of Dislodgeable Carboquat Residue via Wet Hand Rubbing

Days after treatment	Amount of dislodgeable residue after applying a TRF (ug/cm^2)	Standard Deviation (ug/cm^2)
8	0.815	0.719
14	0.457	0.237
21	0.195	0.141
28	0.195	0.126
35	0.136	0.043

Agency Comments:

- The study did not provide details on the treatment process, except that it was conducted at a commercial wood treatment facility using standard practices.
- The study report did not provide an explanation of the percent of carboquat used in the ACQ testing solution with respect to the nominal retention rate; therefore, it is uncertain if the solution was prepared correctly to achieve the nominal retention rate.
- The study did not provide the storage/shipping conditions between the treatment facility and the test site, which was over the span of seven days.
- It is indicated that the analysis and shipment of the wood in this study was not conducted in accordance with GLP standards, but did follow standard commercial practices of another quality control standard.
- The submission does not include any data or discussion for the treatment/sampling of heartwood species. The Agency requests that this is submitted as soon as possible.

MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:

An ammoniacal copper quat (ACQ) formulation containing carboquat and copper was used to pressure treat the wood, and was formulated from Q50-C (manufacturer's end use product). The chemical name of carboquat is Didecyldimethylammonium Carbonate/Bicarbonate (bicarbonate and carbonate are counter ions each with their own CAS numbers; 148812-65-1 and 148788-55-0 respectively). The test substance can also be referred to as Carboquat WP, Carboquat WP-50 or Q50-C.

Analysis of duplicate samples of the test material indicates an average of 52.14% ai (batch #: N4224297). The reference standard was certified to have 49.2% ai (total quaternary) (batch # 1227-63). The manufacturing use product was used as a reference standard because a pure analytical standard for carboquat was not available.

2. Relevance of Test Material to Proposed Formulation(s):

The test product used in this study was Q50-C, which is the manufacturer's end use product for Lonza's Carboquat product registered as Carboquat WP-50. According to the Study Author, Q50-C is of identical composition to both Carboquat WP-50 and the reference standard (Carboquat WP) used in this study.

The Agency accepts this as the test material because the test product is representative of what manufacturers will use to treat wood products to be distributed on the market.

B. STUDY DESIGN:

The study was conducted in accordance with the Centre for Toxicology Study Plan No. 2005-CT-ACQ-PS entitled, "Determination of Dislodgeable Carboquat Residues from Sapwood Boards Pressure Treated with an Ammoniacal Copper Quat (ACQ) Formulation." The Study Director signed this protocol on August 30, 2005.

There were four amendments to and two deviations from the study protocol that was followed for this data collection.

The amendments to the protocol include:

(1) Additional QC samples at higher carboquat concentrations may be added in an attempt to bracket the anticipated carboquat residues dislodged from treated lumber on any given sampling interval;
(2) Sampling events which are delayed due to inclement weather need only to be postponed until conditions are appropriate for sampling. A suitable postponement interval for sampling (or resampling) will be determined at the discretion of the Study Director with the provision that the wood must be visibly dry at the time of sampling;
(3) The MDL and LOQ values reported in the protocol were corrected. The amendment also allows for verification of the method extraction and analysis at a fortification level that brackets the highest dislodgeable carboquat resides concentrations observed during the sampling period; and
(4) The active ingredient of carboquat was clarified to show that it contains both types of counter ions (bicarbonate and carbonate). The deviation from the study protocol was that the treated and untreated wooden boards were shipped three days after treatment instead of within two days after treatment.

The deviations to the protocol include:

(1) The analytical method states that the QC samples should be air dried for 15-20 min after fortification. Some samples were spiked with 1.7mL of the aqueous fortification solution and needed to be air dried for a longer duration to ensure complete dryness. Carboquat is a nonvolatile organic salt molecule and therefore the extended drying period is not expected to affect the amount detected, and
(2) This is the same as the prior deviation, and this extended drying time was needed because of the elevated levels of the larger volume spiked samples to ensure that the wipe samples were completely dry.

The Agency agrees with the registrant that these amendments and deviations do not impact the validity of the data collected in the study.

Surface(s) Monitored:

According to the Study Protocol, SYP is representative of the type of wood used to construct above ground recreational structures. The sizes of the boards sampled in the study were 12 feet x 6 inches x 2 inches.

The Agency requests that the date pertaining to the heartwood species is also submitted. It was agreed prior to initiating the study that both sapwood and heartwood species were to be sampled because both wood types have the potential to be treated and distributed in the market.

Application Rates and Regimes:

Application Method: The boards were pressure-treated at a commercial wood treatment facility, and they

were treated in 3 batches of 6 boards each. According to the study author, wood treatment followed standard commercial practices and was observed by the Sponsor's Representative and documented by the treatment facility. A detailed description of the treatment process was not provided in the Study Report. Shipment to the testing facility occurred three days after treatment.

Application Rate: The boards were treated with an ammoniacal copper quat (ACQ) formulation prepared with Q50-C (Manufacturing End Product containing carboquat) and copper at a preservation concentration intended to yield a nominal quat retention rate of approximately 0.25 pounds per cubic foot (pcf). According to the Study Protocol, 0.25 pcf is representative of retention rates for lumber used to construct above ground recreational structures. The ratio of copper to carboquat in the ACQ formulation was 2:1. Analysis of the ACQ treatment solutions collected during pressure treatment of the SYP boards showed average carboquat concentrations of 0.29, 0.32, and 0.34% for each of the three treatment batches used.

The Agency believes the treatment procedures presented and followed in this protocol are an accurate representation of what will be carried out by manufacturers that use this product. The study report does not provide an explanation of the relationship between the percent carboquat and the retention rate achieved. Verification that the percentages of carboquat are representative of the 0.25pcf retention rate would be useful.

Test Site Description:

The sampling was conducted at the Centre for Toxicology, University of Guelph in Ontario, Canada. After receipt of the treated wooden boards at the test location (7 days after treatment), the boards were unwrapped, labeled, and stacked horizontally on wooden shims (0.2 to 0.5 inch thick) to allow air to circulate under each board (the boards were kept in the greenhouse for the first sampling interval because of the possibility of rain, but were placed outside thereafter as described below). The board surfaces, which did not contact the wrapping materials, were stacked facing upward towards the ceiling. Similar handling procedures and storage conditions were also applied to the untreated wooden boards. The treated and untreated boards were sufficiently separated to avoid potential cross contamination.

When the boards were moved outside, they were laid horizontally across two sawhorse stands (29 to 31 inches above ground). Two boards spaced approximately 0.5 to 1.0 feet apart occupied each pair of sawhorse stands. The boards were secured with one-inch screws from the underside to metal brackets, which had been attached to the sawhorse stands. Additionally, the sawhorse stands were secured to the ground using cinder blocks. Weed barrier sheets were placed on the ground to prevent weeds from growing.

The storage conditions during the seven days between treatment at the commercial pressure treatment facility and arrival at the testing facility were not reported. In addition to this, a storage stability analysis was not conducted. The Agency is not certain as to whether or not there was no change in the level of carboquat on the treated wood as a result of the time elapse between treatment and sampling. However, based on the fortification data, the Agency does believe that Carboquat is indeed stable when refrigerated.

Mean, minimum and maximum air temperature and precipitation (cumulative rainfall) were monitored daily at the outdoor sampling site according to standard operating procedures developed by University of Guelph. Daily weather conditions were also qualitatively assessed and recorded. During the sampling period, the average temperatures ranged from 11 to 24^{o}C and precipitation occurred on 8 of the 28 sampling days (0.3 to 42.1 mm). No rain days were encountered on the designated sampling days. A full report of daily temperatures, precipitation and qualitative weather profiles are provided in the study report.

Dislodgeable Residue Sampling Procedures:

The wipe technique used in this study is modeled after the United States Consumer Product Safety Commission memorandum under the subject heading of CCA-Treated Wood Field Study – Phased III and IV, dated 2003. In the supplemental study, MRID #467640-02, which compared the transferability of carboquat to hands (wet and dry) and polyester wipes (wet and dry), the **dry polyester wipes** produced the most consistent rubbing method and had the lowest sample variability (coefficient of variability of 19.6%).

The study consisted of three batches of treated and untreated boards. Each batch had three treated replicates and one untreated replicate; each replicate consisted of two boards (total of 18 treated boards and 6 untreated boards). Each board was partitioned into 5 equal sections from which wipe samples were collected (total of 10 equal sections per board pair). Each sampling area was 8.0 cm x 62.5 cm (500 cm^2). There was a 5 to 10 cm gap between the edge of the wood and the neighboring sampling area. A wooden template with dimensions of approximately 8.3 cm x 62.5 cm was placed horizontally on the delineated area to be sampled and the template was secured with clamps to the board. The width of the template was slightly wider than the 8 cm track in order to accommodate the thickness of the tape used to secure the wipe on the aluminum block.

The wipe sample material was cut into approximately 9 cm x 9 cm pieces of polyester cloth (Tx 1009 Alpha Wipe). Each wipe was stretched smoothly over the bottom and lower sides of a 1.1 kg aluminum block with bottom surface dimensions of 8 cm x 8 cm. The wipe was then secured to the sides of the block using masking tape, and a clean piece of Parafilm® was placed between the aluminum block and the polyester wipe to prevent contamination from the block. Strings were attached to each side of the block to allow it to be pulled smoothly across the wood surface from all four directions. The dry weight of a typical wipe with dimensions of 8 cm x 8 cm, equivalent to the dimension following removal from the block after sampling was approximately 1.1 grams.

The wipe-covered aluminum block was placed on one end of the template demarcation area and pulled back and forth for five strokes (ten passes) at a slow, constant pace. The block was then rotated 90 degrees and the procedure was repeated for an additional five strokes. This resulted in a total of 20 passes of the wipe over the 500 cm^2 sampling area. At each sampling interval, three replicates were collected from each of the three treatment batches. Therefore, a total of nine replicates were sampled per interval. Sampling occurred on days 8, 14, 21, 28, and 35 after the treatment at the commercial facility. Eight days is considered the shortest time period after pressure treatment that human contact is expected to occur.

The Agency agrees with the sampling procedures.

Sample Handling:

After sample collection, the wipe was cut at all four sides from the bottom surface of the block. The wipe was then placed in an amber bottle containing 30 mL of ethyl acetate and 0.5 mL of 40% aqueous tetramethylammonium chloride (TMAC) as the first extraction step. The samples were stored in the refrigerator (2 – 8^oC) until further extraction and analysis as indicated on page 25 of the submission.

The submitted data does not include any storage stability analysis. The study submission does not indicate how much time elapsed between sample collection and sample analysis. The study also states, "the samples will be extracted an analyzed within 14 days of collection, thus storage stability analyses will not be necessary" (page 56 of 118). The Agency does believe that carboquat is stable in refrigerated conditions but requests confirmation of this from the registrant.

Analytical Methodology:

Carboquat was extracted three times with ethyl acetate in the presence of 40% aqueous TMAC. The ethyl acetate extracts were evaporated to dryness and the carboquat residues were re-dissolved in chloroform:methanol (5:1) in the presence of an internal standard (DoDAB). The chloroform:methanol extracts were analyzed using High Performance Liquid Chromatography (HPLC) equipped with an evaporative light scattering (ELSD) detector. See Table 2 for the typical HPLC operating conditions.

Table 2. Typical HPLC Operating Conditions

Column	HPLC column, normal phase, YMC Polyvinyl alcohol functionalized silica (150 x 4.6 mm, 5 μm, Water Corporation) Guard column: YMC Polyvinyl alcohol functionalized silica S5 120 A (4.0 x 23 mm Threaded Guard, Waters Corporation)
Solvents	Solvent A: 60 mL methanol (optima) 250 mL chloroform (optima) 697 mL hexane (optima) 1 mL triethylamine (TEA) 1 mL formic acid Solvent B: Methanol
Injector volume	20 μL
Detector Conditions	Temperature: 85°C Airflow rate: 1.8 L/min Time Constant: 1 second Impactor: Off
Time Program	The compound of interest is eluted under isocratic conditions at a flow rate of 1.40 mL/min with 100% mobile phase A for 10 minutes. To clean the column, the flow rate is increased to 2.00 mL/min with 100% mobile phase B for 3 minutes. From 13 to 19 minutes the mobile phase is switched to 100% Solvent A to equilibrate the column while maintaining the flow rate at 2.0 mL/min. At 19.01 minutes, the flow rate is reduced to the initial flow rate of 1.40 mL/min

Prior to the commencement of the study, the method was validated on dry polyester wipes as presented in the discussion of MRID #467640-03. Due to unexpectedly high carboquat residues measured at the start of this study, additional method validation was performed during the course of this study using the highest observed carboquat residues measured from the wipe treated boards (2,071 x LOQ). The average recovery was 95.8±2.1%

A standard curve was generated from a series of four to six standards which contained carboquat (certified Manufacturing Use Product (MUP) supplied by the Sponsor), a known volume of chloroform:methanol (5:1), and the internal standard (DoDAB) at a concentration of 200 ppm. The MUP was used because a pure analytical standard for carboquat was not available. The standard curve was generated by plotting the area of carboquat/ DoDAB obtained from the chromatograms versus the corresponding carboquat concentration. The r^2 values presented in the Study Report were greater than 0.98 for all of the curves generated.

Carboquat concentrations in the sample extracts were determined using the regression equation obtained from a series of standards. The amount of carboquat recovered was then calculated by multiplying its concentration (μg/mL) by the final volume of the extract (mL) and appropriate dilution factor (if any).

There are no graphical standard curves provided in this report. However, the standard curve data is provided in the raw data. The R^2 values for each respective day after treatment sampling interval are all > 0.987, therefore the standard curve data are acceptable.

Quality Control:

One sample of the test substance Q50-C (the actual product that the label will be placed on, Reg #6836-236), **which was used to prepare the ACQ treating solution**, was analyzed in duplicate. The samples contained 51.99% and 52.29% carboquat (average of 52.14% carboquat). This is very close to the percent active ingredient on the proposed label, which is 50 %.

One sample of the ACQ treatment solution was collected during the pressure treatment of each batch at the treatment facility and analyzed in duplicate. The average percent carboquat in the three treatment solutions used was 0.32% (batch 1), 0.34% (batch 2), and 0.29% (batch 3).

There is no discussion of the correlation between the percent carboquat detected in the ACQ solution and the retention rate of 0.25pcf.

Laboratory recovery samples were not analyzed; however, standards containing an internal standard at a known concentration and the primary standard were run with each batch of samples analyzed. In addition, a single sample from an untreated wooden board was randomly collected at each sampling interval for each batch of wood, and carboquat was not detected in any of these samples.

Field fortification samples were prepared in duplicate at two fortification levels at each sampling interval, with the exception that only one fortification level was prepared in duplicate on 8 DAT. The low fortification level was 20 to 25 times the LOQ (LOQs are presented in the DER of MRID # 467640-03) and the high fortification level was set at concentrations based upon the results of the previous sampling interval in an attempt to bracket the anticipated carboquat residues dislodged from the treated lumber. Within each duplicate set, one sample was prepared before the sampling start and the other was prepared at the end of the sampling day. Fortification samples were prepared by placing a known amount of carboquat solution over a polyester wipe in a drop-wise fashion. The fortified wipe was then air-dried to evaporate the solvent, and then ethyl acetate and 40% aqueous TMAC was then added to the jar as the first extraction step. The sample was stored in the refrigerator until further extraction and analysis.

While the time elapse between extraction, storage, and analysis were not provided, the Agency believes that the percent recoveries do reflect the stability of carboquat via refrigeration.

Table 3 summarizes the field fortification recoveries provided for this report, along with the average percent recovery for a given day (at either a low or high fortification level) and the overall average percent recovery for a sampling interval (both low and high fortification levels combined).

Table 3. Field Fortification Summary

Sampling Interval (Days After Treatment)	Timing	Fortification Level	Amount Fortified (µg)	Amount Recovered (µg)	Percent Recovery	Average Percent Recovery per Level per Day	Overall Average Percent Recovery per Day
8	Before sampling	Low	181.1	196.9	108.7	107.8	107.8
	After sampling		183.4	196	106.9		
14	Before sampling	Low	179	189.4	105.8	103.0	103.3
	After sampling		187.8	188.3	100.3		
	Before sampling	High	3,042.9	3,285.2	108.0	103.5	

Table 3. Field Fortification Summary							
Sampling Interval (Days After Treatment)	Timing	Fortification Level	Amount Fortified (µg)	Amount Recovered (µg)	Percent Recovery	Average Percent Recovery per Level per Day	Overall Average Percent Recovery per Day
	After sampling		3,192.7	3,164.7	99.1		
21	Before sampling	Low	181.1	181.1	100.0	99.5	101.3
	After sampling		186.7	184.7	98.9		
	Before sampling	High	1,086.5	1,139.6	104.9	103.2	
	After sampling		1,120.2	1,137.4	101.5		
28	Before sampling	Low	182.7	185.1	101.3	108.9	108.9
	After sampling		174.9	203.9	116.6		
	Before sampling	High	639.4	686.9	107.4	108.8	
	After sampling		612.2	674.3	110.1		
35	Before sampling	Low	172.7	185.1	107.2	105.2	102.0
	After sampling		175.5	181.2	103.2		
	Before sampling	High	518.2	547.5	105.7	98.7	
	After sampling		526.5	483	91.7		

The Agency believes that due to the high percent recoveries, that the stability of carboquat via refrigeration is justified even though a storage stability analysis is not provided. The percent recoveries can be assumed representative of the stability of Carboquat in refrigerated conditions.

II. RESULTS AND CALCULATIONS

The carboquat residues were presented in terms of ug/cm^2, and were calculated based on the 500 cm^2 area of the sampled wood surface, and are presented in Table 4. The residues provided in the study report were corrected for method validation and these are the values that are presented in this DER. Although it is common procedure that if the average recoveries are >90%, the values are not corrected, this was not carried out. For all of the values in this table, the method validation overall average recovery used was 93.4%. No residues were less than the LOQ as reported in MRID # 467640-03.

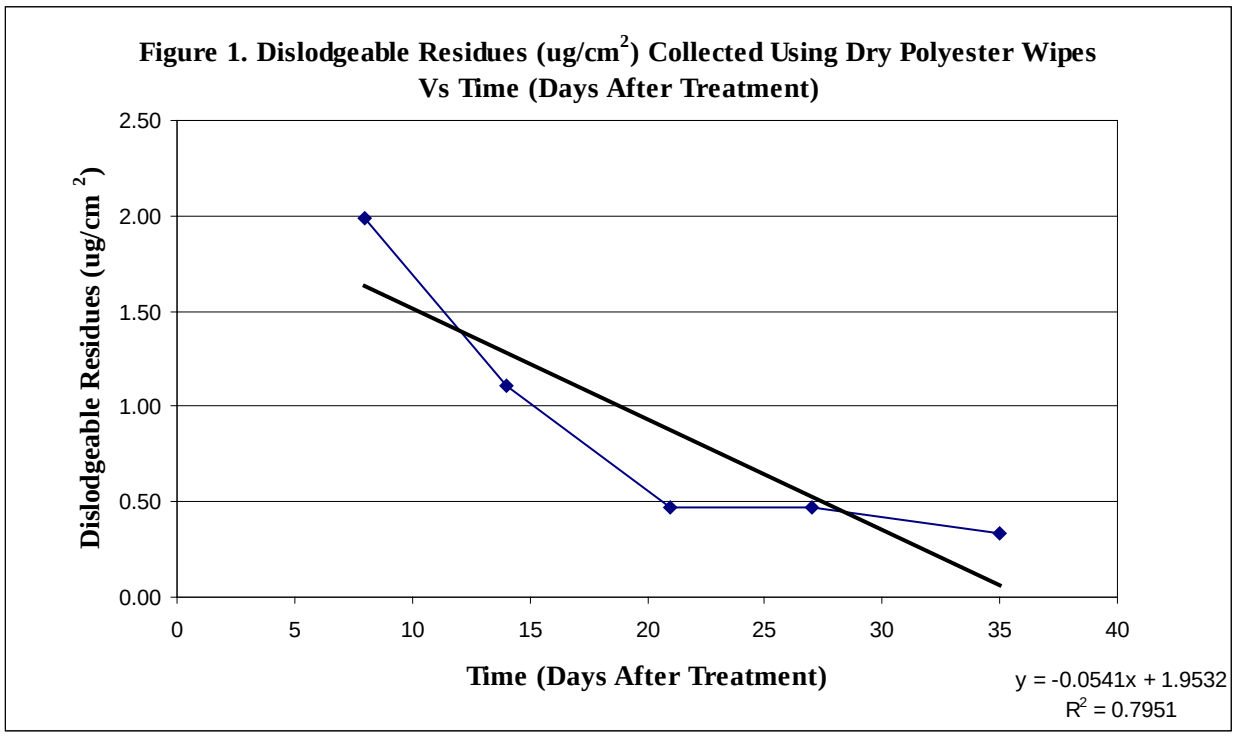
Table 4. Dislodgeable Carboquat Residues from Pressure Treated Sapwood Using Dry Wipe Sampling						
Treatment Batch	Replicate No.	Days After Treatment				
		Day 8	Day 14	Day 21	Day 28	Day 35
Dislodgeable Residue (µg/wipe)						
1	1	630.81	466.01	613.6	118.2	229.9
	2	1621.55	348.07	193.3	508.3	194.9
	3	549.77	1069.27	177.9	461.4	123.1
	Average	934.04	627.78	328.2	362.6	182.6
	Standard Deviation	596.78	386.86	247.2	213.0	54.4
2	1	1105	888.90	350.5	257.7	240.8
	2	740.66	585.17	184.0	245.7	201.6
	3	3052.26	746.88	328.0	210.2	117.6
	Average	1633	740.32	287.5	237.8	186.7
	Standard Deviation	1243	151.97	90.4	24.7	62.9

3	1	545.15	341.80	129.6	98.4	97.9
	2	252.35	310.37	83.4	148.0	140.7
	3	423.33	245.48	69.9	87.3	142.2
	Average	406.94	299.22	94.3	111.2	126.9
	Standard Deviation	147.09	49.12	31.3	32.3	25.2
Overall	Average	991	555.77	236.7	237.2	165.4
	Standard Deviation	874	288.43	171.1	153.6	52.2
Dislodgeable Residue ($\mu g/cm^2$)[a]						
1	1	1.26	0.93	1.23	0.24	0.46
	2	3.24	0.70	0.39	1.02	0.39
	3	1.10	2.14	0.36	0.92	0.25
	Average	1.87	1.26	0.66	0.73	0.37
	Standard Deviation	1.19	0.774	0.49	0.43	0.11
2	1	2.21	1.78	0.70	0.52	0.48
	2	1.48	1.17	0.37	0.49	0.40
	3	6.10	1.49	0.66	0.42	0.24
	Average	3.27	1.481	0.57	0.48	0.37
	Standard Deviation	2.49	0.304	0.18	0.05	0.13
3	1	1.09	0.684	0.26	0.20	0.20
	2	0.505	0.621	0.17	0.30	0.28
	3	0.847	0.491	0.14	0.17	0.28
	Average	0.814	0.598	0.19	0.22	0.25
	Standard Deviation	0.294	0.098	0.06	0.06	0.05
Overall	Average	1.98	1.11	0.47	0.47	0.33
	Standard Deviation	1.75	0.577	0.34	0.31	0.10

a. Dislodgeable residues were calculated based on the 500 cm^2 area of the sampled wood surface.

To determine if there were any significant differences in the dislodegeable carboquat residues between the treatment batches, the study presents the results of the Tukey tests conducted on the data for each of the sampling days. The Tukey tests indicated that there were no statistical differences between the treatment batches; therefore the treatment batch replicates are comparable for analysis.

The average residues ± standard deviation for all three treatment groups combined were (provided in Table 4) plotted. A graph using this data to show the decline of the average residues over time is shown as Figure 1. A pseudo-steady state was achieved by 21 days after treatment.



The dry polyester wipe residue data were transformed in the study to represent residues that would transfer from the pressure treated wood boards to wet hands. The calculations were conducted by dividing the Carboquat residue on the dry polyester wipes by a transfer reduction factor calculated from the study "Comparison of Dislodgeable Residues from Carboquat Pressure Treated Lumber using Hand Rubbing Versus Rubbing with Dry Polyester Wipes" (MRID 467640-02). A transfer reduction factor is the ratio of carboquat residues dislodged by dry polyester wipes relative to that dislodged by hand rubbing and was calculated to be 2.43. After transforming the data for wet hands, the average dislodgeable residues ± standard deviation for all three treatment groups combined were calculated and are summarized in Table 5. These values calculated are consistent with the values that were provided in the study report.

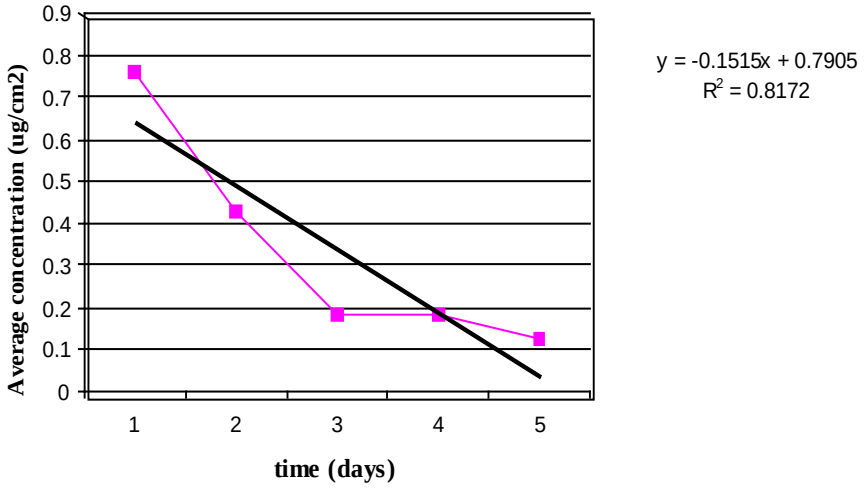
Table 5. Dislodgeable Residues (µg/cm^2) Adjusted for Wet Hand Transfer Reduction Factor

Treatment Batch	Replicate No.	Days After Treatment				
		Day 8	Day 14	Day 21	Day 28	Day 35
Dislodgeable Residue (µg/cm^2)						
1	1	0.519	0.384	0.505	0.097	0.189
	2	1.335	0.286	0.159	0.418	0.160
	3	0.452	0.880	0.146	0.380	0.101
	Average	0.77	0.517	0.270	0.298	0.150
	Standard Deviation	0.491	0.318	0.203	0.175	0.045
2	1	0.909	0.732	0.288	0.212	0.198
	2	0.610	0.482	0.151	0.202	0.166
	3	2.512	0.615	0.270	0.173	0.097
	Average	1.344	0.609	0.237	0.196	0.154
	Standard Deviation	1.023	0.125	0.074	0.020	0.052
3	1	0.449	0.281	0.107	0.081	0.081
	2	0.208	0.255	0.069	0.122	0.116

	3	0.348	0.202	0.058	0.072	0.117
	Average	0.335	0.246	0.078	0.092	0.104
	Standard Deviation	0.121	0.040	0.026	0.027	0.021
Overall	Average	0.816	0.457	0.195	0.195	0.136
	Standard Deviation	0.719	0.237	0.141	0.126	0.0430
	Coefficient of Variation (%)	88.2	51.9	72.3	65	31.6
	90th Percentile	1.57	0.761	0.332	0.387	0.191
	75th Percentile	0.909	0.615	0.270	0.212	0.166
	Median	0.519	0.384	0.151	0.173	0.117
	Maximum	2.51	0.880	0.505	0.418	0.198
	Count	9	9	9	9	9

The Agency also performed a linear regression of the average carboquat concentrations as presented in Figure 2, and this was done using the overall average values from Table 5. The data in Table 5 takes into account the transfer reduction factor into the numerical calculations. The r^2 value is fairly high at 0.8172.

Figure 2: <u>Average</u> Carboquat Concentrations over a 8-35 DAT period



III. <u>CONCLUSION</u>

The average transformed dislodgeable residues for wet hands presented by the study author are the same as those calculated in this DER. The study author concluded that the dislodgeable residue reached a steady state after approximately 21 days post-treatment at a concentration of 0.19 μg/cm^2 of treated wood when corrected for a wet hand transfer reduction factor. Additionally, the study author concluded that an equilibrium plateau was reached by 35 days post-treatment. The Agency agrees with these conclusions.

Minor issues within the study report, but which are not expected to have a significant impact on the quality of the data provided are:

- The study was only conducted during a single time frame and in only one geographically distinct

area. It is unknown if the meteorological conditions during the study represented worst-case or typical weather conditions.

- The study did not provide details on the treatment process, except that it was conducted at a commercial wood treatment facility using standard practices.
- The study report did not provide an explanation of the percent of carboquat used in the ACQ testing solution with respect to the nominal retention rate, therefore, it is uncertain if the solution was prepared correctly to achieve the nominal retention rate.
- The nominal retention rate was not verified through sampling and analysis.
- The study did not provide the storage/shipping conditions that occurred over the course of the seven days between treatment and arrival at the test site.

If this information is available, the Agency requests that it is submitted, along with the data for the heartwood species.

DATA EVALUATION RECORD: "Comparison of Dislodgeable Residue from Carboquat Pressure Treated Lumber Using Hand Rubbing versus Rubbing with Wet and Dry Polyester Wipes" (MRID 467640-02)

EXECUTIVE SUMMARY:

This study was designed to determine the difference in the amount of carboquat that can be dislodged from the surface of pressure treated wood by rubbing the surface of a board with either a wet or dry bare palm of the hand versus wet or dry polyester wipe. This data was used to determine the most appropriate surrogate material to use in MRID #467640-01, "*Determination of Dislodgeable Carboquat Residues from Sapwood Boards Pressure Treated with an Ammoniacal Copper Quat (ACQ) Formulation*." The selection of the material and whether or not it was to be wet or dry was based on which one was concluded to quantify dislodgeable residues the most consistently. The data collected in this study was also used to develop transfer reduction factors based on the ratio of carboquat residues dislodged by hand rubbing relative that dislodged by wipes.

This study was conducted using a single batch of sapwood SYP boards that had been pressure treated with an ammoniacal copper quat (ACQ) formulation. The formulation was prepared using the manufacturing end use product Q50-C, and the boards were treated to a nominal preservative retention rate of 0.25 pcf (pounds per cubic foot) following standard commercial practices. Following treatment, wipe samples were collected from the treated boards using the following four different sampling techniques: dry palms, wet palms, dry polyester wipes, and wet polyester wipes. Duplicate treatment samples and a single untreated sample were collected on four different days for each sampling method (total of 8 treatment replicates and 4 untreated replicates collected for each sampling method), and each sample was collected from a surface area of 500 cm^2.

Although common practice is that the Agency does not correct for values with a recovery >90%, all the values provided in the study were corrected. The data show that the dry palm sampling method dislodged the least amount of residue and had the highest sample variability. The wet wipe sampling method dislodged the most amount of residue. However, the dry wipe sampling dislodged the second to most amount of residue and had the least sample variability for the entire selection of wipe methods employed. Due to this consistency, the dry wipe was the methodology employed in MRID #467640-01, "*Determination of Dislodgeable Carboquat Residues from Sapwood Boards Pressure Treated with an Ammoniacal Copper Quat (ACQ) Formulation*."

Transfer reduction factors (ratio of the average wipe residue to the average palm residue) calculated show that dry wipes overestimate dislodgeable carboquat from treated lumber 2.43 times relative to wet hands.

The amendments and deviation to the study protocol are provided below. However, the Agency believes that these will not significantly affect the conclusions drawn by the data collected in this study because the study was more qualitative in nature and served the purpose of selecting a methodology to be used in MRID # 467640-01.

- The study did not verify the retention rate after treatment of the wood, the percent carboquat in the test substance formulation, or the percent carboquat in the tank mix.
- The time between board treatment and sample collection was not provided nor the storage/shipping conditions between the treatment facility and the testing facility.
- The study did not provide details on the treatment process, except that it was conducted at a commercial wood treatment facility using standard commercial practices.

The Agency does request that any heartwood species data that the sponsor has on file is provided. It was discussed and agreed that data would be collected from both sapwood and heartwood species because both wood types have the potential to be distributed in the market.

I. MATERIALS AND METHODS

A. MATERIALS

Like in the primary study, MRID #467640-01, the same chemicals were used for this study. The amount of the a.i. in the test material, Q50-C (batch #: D5224954), was not provided. The reference standard (batch #: 1227-63, same batch as used in MRID # 467640-01 was certified to have 49.1% a.i. (total quaternary)

B. STUDY DESIGN

The study was conducted in accordance with the Centre for Toxicology Study Plan No. 2005-CT-HWD entitled, "Comparison of Dislodgeable Residues from Carboquat Pressure Treated Lumber Using Hand Rubbing versus Rubbing With Wet and Dry Polyester Wipes". The Study Director signed this protocol on May 17, 2005.

There was one amendment to the study protocol, which clarified that the active ingredient carboquat contains both types of counter ions (bicarbonate and carbonate). The three deviations to the study protocol include:

(1) Untreated test board K, which was mistakenly designated for the wet palm rubbing area, was incorrectly sampled using the left palm that was used to rub the dry palm area. To correct the error, a new untreated control board, which was provided as an extra control board by the Sponsor, was sampled for the wet palm;
(2) During sample preparation, sample DPF6 (dry palm rubbing sample) was lost due to water contamination and loss of sample. The loss of one sample in the dry palm set provided seven replicates in total. The Study Author noted that the difference in standard error associated with N=7 versus N=8 is 6.4%; and
(3) the sample locations on board H were adjusted to prevent potential splintering in the human volunteers' hands. According to the Study Director, none of the protocol amendments or deviations impacted the validity of the study.

There were also two deviations from the analytical protocol. These include:

(1) For the analysis of the untreated board (sample WWI1), the residue was mistakenly reconstituted in 2 mL instead of 1 mL. For consistency and to determine if a carboquat peak was present in 1 mL of extract, a 1-mL subsample was reconcentrated, evaporated to dryness and reconstitued with 0.5 mL of 5:1 chloroform:methanol containing 200 ppm of didecyldimethylammonium bromide (DoDAB) internal standard. As a result, the concentration of DoDAB was incorrectly enhanced by a factor of three. No peak was detected; and
(2) All palm rubbing sample extracts were filtered through acid-washed glass wool prior to transfer to HPLC vials to remove particulates. The Study Author noted that adequate percent recoveries were observed in the quality control samples. Additionally, all palm sample extracts in the method validation and method development were filtered through acid-washed glass wool prior to transferring to HPLC vials. According to the Study Director, none of the analytical deviations impacted the validity of the study.

The Agency agrees with the study director that all of the amendments and deviations mentioned do not affect the quality and validity of the data collected in this study.

Surface(s) Monitored:

Each SYP board that was used measured 10 feet x 6 inches x 2 inches. There were no details provided for the surface condition of the wood.

The Agency requests that the data for heartwood species is submitted as well. It was agreed upon that data for both types of wood species would be collected.

Application Rates and Regimes:

The application method and application rates, as well as the information reported in this MRID were the same as what was reported in MRID #467640-01. The only difference is that the ACQ treatment solutions were not collected and sampled to verify the average carboquat concentrations.

The Agency accepts that the ACQ treatment solutions were not collected and sampled, because it appears that the treatment process carried out in this study is the same as to what was carried out in MRID #467640-01.

Test Site Description:

The sampling was conducted at the Centre for Toxicology, University of Guelph in Ontario, Canada. The boards were stored and set up the same way as reported for MRID # 467640-01, with the only exception that they were indoors, not outdoors.

Like MRID # 467640-01, the storage conditions and the time elapsed between treatment at the commercial pressure treatment facility and the arrival at the testing facility were not reported. The indoor meteorological conditions during storage and sampling, such as room temperature and humidity, were not discussed in the Study Report.

Dislodgeable Residue Sampling Procedures:

Four techniques were used to collect samples including dry and wet palm rubbing and dry and wet polyester wipe rubbing. The techniques are modeled after the United States Consumer Product Safety Commission memorandum under the subject heading of CCA-Treated Wood Field Study – Phased III and IV, dated 2003. There were eight replicates per sample type and the sampling area was 500 cm^2. The boards were sampled on Days 1, 3, 8, and 10. On each day, two replicates for each sample method were collected.

The study consisted of eight treated boards and five untreated boards. For sampling purposes, the wooden boards were placed on saw-horse stands (treated and untreated board were kept separate to avoid cross contamination). Care was taken to avoid touching the areas of the wood surfaces during moving and placing of the boards on the saw-horse stands. Each board was partitioned into four equal areas for dry hand rubbing, wet hand rubbing, dry wipe rubbing, and wet wipe rubbing. The untreated boards were used for control blanks. Two 500 cm^2 areas (8 cm x 62.5 cm) for the dry and wet hand rubbing areas were measured on one half of each board and marked with masking tape to produce an 8-cm wide track along the top surface of each board. There was an approximately 8 to 10 cm gap between the two sampling areas. The remaining half of the board was divided into two equal-length tracks that were marked using a pencil. These tracks were used for dry and wet polyester wipe rubbing areas. These areas were big enough for 8.3 cm x 62.5 cm templates.

Dry and Wet Palm Rubbing: Human volunteers were used to collect the palm rubbing samples. Eight volunteers each were used for the dry and wet rubbing of the treated wood and four volunteers each were used for the dry and wet rubbing of the untreated wood. Two volunteers were also used to collect the dry hand field fortification samples. Each volunteer used one hand only. No personal information on the volunteers such as age, gender, or area of palm was provided in the Study Report.

The volunteers participating in the dry wipe sampling washed their hands with soap and water, rinsed with copious amounts of tap water and then rinsed a second time with 100 mL of deionized water. The hands were then dried with a paper towel followed by further drying with the aid of a gently blowing fan for 2 to 5 minutes. The bare palm of the volunteer was placed on the board so that the palm of the hand covered the width of the designated sampling area. Care was taken to prevent contact of the fingers with board. A 1.1 kg aluminum block was placed on top of the hand during the rubbing procedure in order to maintain a constant pressure (the investigator helped to hold the block from sliding off the hand while avoiding putting pressure on the hand). The participant rubbed with a slow and constant pace on the designated area section for 10 strokes (20 passes). A stroke was considered to be one forward and back movement along the designated track. The residues on the hand were removed by wiping the surface of the palm four times with wipes dampened with a mixture of 70% rubbing alcohol:30% deionized water containing 5% acetic acid followed by rinsing the hand with 6 to 8 mL of the same mixture. Each wipe and the rubbing alcohol rinsate were placed in an amber bottle containing 30 mL of ethyl acetate and 0.5 mL of 40% aqueous tetramethylammonium chloride (TMAC) as the first extraction step.

The procedure for wet palm rubbing was the same as described for dry palm rubbing with the exception that after the hand had been dried with a paper towel; the hand was wetted with 0.9% saline (NaCl) solution. To wet the hand, the volunteer squeezed a polyester wipe (approximately 20 cm x 20 cm) that had been wetted with 0.9% saline solution over the sink. Once excess saline solution had been squeezed out (when the wipe stopped dripping), the volunteer commenced rubbing with the wetted (but not dripping) hand.

Dry and Wet Wipe Sampling: The wipe samples were approximately 9 cm x 9 cm pieces of polyester cloth (Tx 1009 Alpha Wipe). The sampling device used to collect the wipe samples was the same as what was used in MRID #467640-01 along with the method of wiping the surface.

A wooden template with dimensions of approximately 8.3 cm x 62.5 cm was placed horizontally on the delineated area to be sampled and the template was secured with clamps to the board. The width of the template was slightly wider than the 8 cm track in order to accommodate the thickness of the tape used to secure the wipe on the aluminum block. The wipe-covered aluminum block was placed on one end of the template demarcation area and pulled back and forth for 5 strokes (1 stroke = 2 passes) at a slow, constant pace along the track of 8.3 cm x 62.5 cm. The block was then rotated 90 degrees and the procedure was repeated for an additional 5 strokes. This resulted in a total of 20 passes of the wipe over the 500 cm^2 sampling area. After rubbing, the wipe was cut at all four sides from the bottom surface of the block. The wipe was then placed in an amber bottle containing 30 mL of ethyl acetate and 0.5 mL of 40% aqueous TMAC as the first extraction step.

The procedure for wet wipe rubbing was the same as described for dry wipe rubbing with the exception that the wipes were wetted with 0.9% saline (NaCl) solution prior to the start of the sampling. Using a pipette, 1.3 mL of 0.9% of saline solution was deposited evenly to the dry wipe. The volume was previously determined to approximately double the weight of an 8 cm x 8 cm dry polyester wipe.

Sample Handling:

The samples were stored in the refrigerator (2 – 8°C) until extraction and analysis. Extraction took place one day after sample collection and analysis took place either on the day of or the day after extraction.

There was no data collected with respect to storage stability, so there is no confirmation that the time elapsed up to extraction and analysis could have allowed for dissipation of the actual carboquat on the wipe when it was initially collected. However, because the samples were stored at freezing, the Agency believes that it is highly unlikely that any dissipation could have occurred.

Analytical Methodology:

The analytical methodology employed was the same as what was used for the analysis of the data in MRID #467640-01 and justified in MRID #467640-03. Prior to the commencement of all of the studies, the method was validated for hands, dry wipes, and wet wipes (as presented in the discussion of MRID #467640-03).

According to the Study Report, a standard curve was generated from a series standards (range: 7.8 – 613.7 µg/mL) which contained carboquat (certified Manufacturing Use Product supplied by the Sponsor), a known volume of chloroform:methanol (5:1), and the internal standard (DoDAB) at a concentration of 200 ppm. The MUP was used because a pure analytical standard for carboquat was not available. The standard curve was generated by plotting the area of carboquat/ DoDAB obtained from the chromatograms versus the corresponding carboquat concentration. The r^2 values were greater than 0.987 for all curves generated.

Carboquat concentrations on the sample extracts were determined using the regression equation obtained from a series of standards. The amount of carboquat recovered was then calculated by multiplying its concentration (µg/mL) by the final volume of the extract (mL) and appropriate dilution factor (if any).

There are no graphical standard curves provided in this report. However, the standard curve data is provided in the raw data. The R^2 values for each respective day after treatment sampling interval are all > 0.987 as reproduced by the Agency; therefore the standard curve data are acceptable.

Quality Control:

Laboratory recovery samples were not analyzed; however, standards containing an internal standard at a known concentration and the primary standard were run with each batch of samples analyzed. In addition, a single sample for each method was collected on each of the four sampling days from an untreated board, and carboquat was not detected in any of these samples.

Throughout the data collection, single field fortification samples were prepared on two of the sample days (first and last day of sampling) for the human hand, dry wipe, and wet wipe sampling methods. Separate trials were not conducted for the dry and wet palms since the palm was wetted by the aqueous fortification solution.

The field recovery results are summarized in Table 6 and these values were extracted from the study report. Individual recoveries ranged from 88.4% to 111.7%. Average recoveries were 97.5% for the human palm, 104.3% for the dry wipe, and 108% for the wet wipe.

Table 6. Field Fortification Summary					
Matrix	**Timing**	**Amount Fortified (ug)**	**Amount Recovered (ug)**	**% Recovery**	**Average % Recovery**
Human Palm	First Day of Sampling	220.1	194.5	88.4	97.5
	Last Day of Sampling	213.6	227.8	106.6	
Dry Wipe	First Day of Sampling	550.3	585.1	106.3	104.3
	Last Day of Sampling	534.1	546.7	102.4	
Wet Wipe	First Day of Sampling	550.3	614.6	111.7	108.0
	Last Day of Sampling	534.1	557.4	104.4	

Storage stability was not discussed in the Report, but it was indicated that the samples were analyzed within two days of collection. The Agency believes that based on the data and percent recovery values that there was no significant change, if any, in the stability of carboquat. It was still detected at high enough levels that the dry wipe methodology could be selected and justified for the wipe study.

II. RESULTS AND CALCULATIONS

The carboquat residues were presented in terms of µg/cm^2 and were calculated based on the 500 cm^2 area of the sampled wood surface. Although it is typical practice of the Agency to not correct for recoveries >90%, the report corrected all of the data collected regardless if it was greater than or less than 90% recovery. For purposes of consistency, the corrected values are what are reported in this DER. For residues <MDL, a value of ½ MDL was used in the calculations. The data, as summarized in Table 7, supports that the dry wipe sampling method dislodged the second most amount of residue and exhibited the least sample variability.

Table 7. Carboquat Residues Dislodged By Dry Palm Rubbing, Wet Palm Rubbing, Dry Wipe Rubbing, and Wet Wipe Rubbing					
Sample Day[1]	**Replicate**	**Carboquat Residue[2] (µg)**	**Correction Factor[3] (%)**	**Corrected Carboquat Residue (µg/hand)**	**Dislodgeability[4] (µg/cm^2)**
DRY PALM RUBBING					
1	1	18.94	78%	24.3	0.049
	2	19.33	78%	24.8	0.050
3	3	50.9	78%	65.3	0.131
	4	ND[5]	NA	5.8	0.012
8	5	19.35	78%	24.8	0.05
	6	Sample Lost	NA	NA	NA
10	7	ND[5]	NA	5.8	0.012
	8	ND[5]	NA	5.8	0.012
Average				22.4	0.045
Standard Deviation				21.1	0.042
Coefficient of Variation (%)				94.5	94.5
WET PALM RUBBING					
1	1	49.05	78%	62.9	0.126

Table 7. Carboquat Residues Dislodged By Dry Palm Rubbing, Wet Palm Rubbing, Dry Wipe Rubbing, and Wet Wipe Rubbing					
Sample Day[1]	**Replicate**	**Carboquat Residue[2] (µg)**	**Correction Factor[3] (%)**	**Corrected Carboquat Residue (µg/hand)**	**Dislodgeability[4] (µg/cm^2)**
	2	37.38	78%	47.9	0.096
3	3	118.34	78%	151.7	0.303
	4	26.88	78%	34.5	0.069
8	5	123.9	78%	158.9	0.318
	6	213.89	78%	274.2	0.548
10	7	70.38	78%	90.2	0.180
	8	17.32	78%	22.2	0.044
Average				105	0.211
Standard Deviation				85.2	0.170
Coefficient of Variation (%)				80.9	80.9
DRY POLYESTER WIPE RUBBING					
1	1	264.57	93.4%	283.3	0.567
	2	272.43	93.4%	291.7	0.583
3	3	282.27	93.4%	302.2	0.604
	4	179.88	93.4%	192.6	0.385
8	5	166.58	93.4%	178.4	0.357
	6	244.54	93.4%	261.8	0.524
10	7	215.56	93.4%	230.8	0.462
	8	289.1	93.4%	309.5	0.619
Average				256	0.513
Standard Deviation				50.3	0.101
Coefficient of Variation (%)				19.6	19.6
WET POLYESTER WIPE RUBBING					
1	1	565.1	94.1%	600.5	1.2011
	2	398.89	94.1%	423.9	0.8478
3	3	930.77	94.1%	989.1	1.9783
	4	405.97	94.1%	431.4	0.8628
8	5	283.18	94.1%	300.9	0.6019
	6	627.01	94.1%	666.3	1.3326
10	7	325.31	94.1%	345.7	0.6914
	8	659.14	94.1%	700.5	1.4009
Average				557	1.115
Standard Deviation				228	0.457
Coefficient of Variation (%)				41.0	41.0

1. Sampling took place on four days, starting on Day 1. The length of time between treatment and sampling was not provided.
2. Residues detected on one palm (wet or dry) or wipe (wet or dry) after rubbing over 500 cm^2 of treated wood.
3. Residues were corrected for all of the values.
4. Dislodgeability (µg /cm^2) = Corrected residue (µg) / surface area of wood rubbed (500 cm^2).
5. ND = Not detected above the MDL (11.6 µg (0.023ug/cm^2) for dry palm rubbing samples). A value of 1/2 MDL was used in the calculations (5.8 µg for dry palm rubbing samples).

Table 8 summarizes the residues on the palms as a percent of the residues on the wipes as presented in the Study Report. The residues on dry palms are approximately 9% and 4% the residue on the dry and wet wipes, respectively. The residues on wet palms are approximately 41% and 19% of the residue on the dry and wet

wipes, respectively.

Table 8. Residues on palm as a percent of the residues on a wipe[1]		
	Dry Wipe	**Wet Wipe**
Dry Palm	8.73	4.01
Wet Palm	41.09	18.90

1. Average Palmer Dislodgeability (µg /cm^2) * 100 /Average wipe Dislodgeability (µg /cm^2)

Table 9 summarizes the transfer reduction factors. These values are the ratio of the average wipe residue to the average palm residue. The data shows that dislodgeable carboquat residues from treated lumber are overestimated by dry wipes by factors of 11.46 and 2.43 times relative to dry and wet hands, respectively, and dislodgeable carboquat residues from treated lumber are overestimated by wet wipes by factors of 24.9 and 5.29 times relative to dry and wet hands, respectively.

Table 9. Transfer Reduction Factor[1]		
	Dry Wipe	**Wet Wipe**
Dry Palm	11.46	24.9
Wet Palm	2.43	5.29

1. Average wipe residue/Average palm residue

III. CONCLUSION:

The Agency believes that the quantification of carboquat as well as the wood species treated was not critical to the purpose of this study, which serves to compare the different candidate wipe for MRID #467640-01. The data presented in this study is acceptable and provides ample justification for the use of a dry wipe in the, “Determination of Dislodgeable Carboquat Residues from Sapwood Boards Pressure Treated with an Ammoniacal Copper Quat (ACQ) Formulation” (MRID #467640-01).

DATA EVALUATION RECORD: "Validation of Methods to Extract and Analyze Carboquat Residues from the Palm of the Human Hand and from Dry and Wet Polyester Wipes" (MRID 467640-03)

EXECUTIVE SUMMARY:

This study was designed to validate methods of extraction and analysis of carboquat (Didecyldimethylammonium Carbonate/Bicarbonate) from three different fortified matrices – the palm of the human hand, dry polyester wipes, and wet polyester wipes. In this method, carboquat was extracted from polyester wipes using a sonication and shaking method with ethyl acetate in the presence of tetramethlammonium chloride (TMAC). Upon removal of ethyl acetate by evaporation, the residue was re-dissolved in chloroform:methanol (5:1) in the presence of the internal standard didodecyldimehtylammonium bromide (DoDAB). The carboquat compounds were analyzed using High Performance Liquid Chromatography (HPLC) equipped with an YMC PVA-Sil column (150- X 4.6 mm, 5 µm) and an Evaporative Light Scattering Detector (ELSD).

For each matrix (hand, wet wipe, and dry wipe) seven samples were fortified at three different levels. Prior to fortification, the human volunteers washed their hands with soap and water, rinsed with water, and dried with a paper towel and a gentle blowing fan. Additionally, to generate the wet wipes, the polyester pieces were wetted with 0.9% saline solution. The fortification solution was applied evenly to each matrix using an Eppendorf pipette. For the dry wipes and hands, the fortified matrices were dried using a gentle stream of air to remove the solvent. Residues were removed from the hands by wiping the palms four times with polyester wipes that had been moistened with a solution of 70% rubbing alcohol, 30% deionized water and 5% acetic acid.

Percent recoveries were calculated for each matrix at different fortification levels and provided in the study report, and are summarized below in Table 10.

Table 10: Percent Recoveries for Various Fortification Levels for Each Sampling Matrix

Matrix	Fortification Level (µg)	Percent Recovery	Average Percent Recovery
Human Palm	58.2	78.4	78.0±5.9%
	231.2	80.7	
	566.6	74.8	
Dry Wipe	24.9	94.7	93.3±4.8%
	256.1	90.8	
	639.8	94.5	
Wet Wipe	25.9	93.5	94.1±3.9%
	253.4	93.5	
	622.0	95.4	

The method detection limit (MDL) and limit of quantitation (LOQ) values were presented in this MRID, #467640-03, and were calculated from using the seven replicates fortified at the lowest level. The MDL values for the palm, dry wipe, and wet wipe matrices are 11.6 µg (0.023 µg/cm^2), 4.2 µg (0.008 µg/cm^2), and 3.2 µg (0.006 µg/cm^2), respectively. The LOQ values for the palm, dry wipe, and wet wipe matrices are 23.2 µg (0.046 µg/cm^2), 8.5 µg (0.017 µg/cm^2), and 6.4 µg (0.013 µg/cm^2), respectively. The values in µg/cm^2 are based on a theoretical surface area of 500 cm^2.

I. MATERIALS AND METHODS

A. MATERIALS

Like in the other two studies, MRID # 467640-01, 02, the same chemicals were used for this study. The amount of the ai in the test material, Q50-C (batch #: D5224954), was not provided. The reference standard (batch #: 1227-63, same batch as used in MRID # 467640-01 was certified to have 49.1% a.i. (total quaternary)

B. STUDY DESIGN

The study was conducted in accordance with the Centre for Toxicology Study Plan No. 2005-CT-MV-HDWR, entitled "Validation of Methods to Extract and Analyze Carboquat Residues from the Palm of the Human Hand and from Dry and Wet Polyester Wipes". This protocol was signed by the Study Director on April 6, 2005.

Additionally, this study was conducted according to the Standard Operating Procedure CT-014b, entitled "Extraction of Didecyldimethyl Ammonium Bicarbonate (carboquat) from Polyester Wipes by Sonication and Shaking and Analysis by HPLC Equipped with an Evaporative Light Scattering Detector (ELSD)." This Standard Operating Procedure was signed by the Study Director on April 4, 2005.

There was one amendment to the study protocol and one deviation from the analytical method. The amendment to the protocol clarified that the active ingredient carboquat contains both types of counter ions (bicarbonate and carbonate). The deviation from the analytical method was that for the analysis of the control blank of the palm of the human hand and polyester wipes; carboquat standards were not run concurrently with the samples. The standards were not run because no quantifiable peaks of carboquat were detected. According to the Study Director, the amendment and deviation did not impact the validity of the study.

The Agency agrees with the study director that all of the amendments and deviations mentioned do not affect the quality and validity of the data collected in this study

Preparation of Fortification Solutions

A primary aqueous stock fortification solution with a nominal carboquat concentration of 0.25% (i.e., 2,500 ppm carboquat) was prepared by appropriately diluting the test substance with deionized water. To prepare a secondary fortification solution (250 ppm), the primary stock fortification solution was diluted 10-fold with deionized water. These fortification solutions were analyzed in duplicate to verify the carboquat concentration each time of use. The average measured concentration of the respective fortification solution was used to determine percent recovery for the extracted samples.

Fortification Procedures

For each matrix, there were seven replicates per each of three fortification levels. In addition, seven control blanks were prepared for each matrix, and carboquat residues were not detected in any of the blanks.

Before treating the human hand, the volunteers washed their hands with soap and water and followed with a rinse with tap water and then with 100 mL of deionized water. The hands were dried with a paper towel (ALL WORKD 02710^{TM}) followed by further drying with the use of a gentle blowing fan for 2 to 5 minutes. To each of seven bare hands, an aliquot of the aqueous fortification solution was deposited evenly using an

Eppendorf pipette in a drop-wise fashion. The fortified solution on each hand was allowed to air dry for 10 to 15 minutes with the aid of a gentle blowing fan. After this time, the surface of the palms of the hands to which the fortification solution was applied was wiped four times with polyester wipes (approximately 4 cm x 4 cm) that had been moistened with a solution consisting of 70% rubbing alcohol, 30% deionized water and 5% acetic acid and then rinsed with 6 to 8 mL of the same solution. These four wipes and rubbing alcohol rinsates from each hand were combined into a labeled screwcap amber glass jar.

For the dry polyester wipe fortifications, each of seven wipes (8 cm x 8 cm) was placed into a labeled amber glass jar. A predetermined amount of carboquat fortification solution was deposited evenly on each wipe using an Eppendorf pipette in a drop-wise fashion. The fortified wipes were dried using a gentle stream of air for 10 to 20 minutes to remove the solvent. A procedure identical to the one used for the dry wipes was used to fortify the wet wipes with the exception that prior to fortification the wipes were wetted with 1.3 mL of 0.9% saline (NaCl) solution. The volume of saline solution was previously determined to approximately double the weight of an 8 cm x 8 cm dry polyester wipe. The wet fortified samples were not dried after fortification.

Extraction Methodology:

To each jar containing a wipe sample, 0.5 mL of 40% aqueous tetramethylammonium (TMAC) was added along with 30 mL of ethyl acetate as an extracting solvent. Each jar was placed for 10 minutes in a sonication bath (first extraction only) containing warm water (40 to 60^{o}C) and then placed in an orbital shaker for 20 minutes. The ethyl acetate was then decanted into a labeled collection flask. The extraction of carboquat from each wipe was repeated 2 more times, each time by adding 0.5 mL if 40% aqueous TMAC and 30 mL of ethyl acetate with shaking for 20 minutes (second extraction) and 10 minutes (third extraction). The ethyl acetate from each extraction step was collected into the appropriate collection flask. By using forceps, each wipe was transferred to a clean funnel. Each jar was rinsed twice with 2 to 3 mL of ethyl acetate and the ethyl acetate rinsates were poured onto the corresponding wipe to allow rinsates to be combined in the collection flask. Each wipe was further rinsed with 5 to 7 mL of ethyl acetate and squeezed to remove solvent and then discarded. Subsequently, the funnel rinsed with an additional 1 to 2 mL of ethyl acetate. The ethyl acetate collected in the collection flask was evaporated to near dryness using a rotary evaporator. The residue was re-dissolved in acetone and then transferred quantitatively to a 15-mL test tube via a funnel containing an acid-washed glass wool plug and acidified sodium sulphate to remove traces of water. The acetone was then evaporated to dryness using a gentle stream of air supplied by a nitrogen evaporator. Once dry, the carboquat residue was re-dissolved in a known volume of 5:1 chloroform:methanol containing 200 ppm DoDAB (didecyldimethylammonium bromide) as an internal standard. Prior to transferring the final extract to a labeled HPLC vial for analysis, the sample was sonicated for at least 5 minutes followed by vortexing for at least 1 minute.

Detection Methodology:

The chloroform:methanol extracts were analyzed using High Performance Liquid Chromatography (HPLC) equipped with an Evaporative Light Scattering Detector (ELSD). See Table 11 for the typical HPLC operating conditions.

Table 11. Typical HPLC Operating Conditions	
Column	HPLC column, normal phase, YMC Polyvinyl alcohol functionalized silica (150 x 4.6 mm, 5 µm, Water Corporation) Guard column: YMC Polyvinyl alcohol functionalized silica S5 120 Δ(4.0 x 23 mm Threaded Guard, Waters Corporation)
Solvents	Solvent A: 60 mL methanol (optima)

Table 11. Typical HPLC Operating Conditions	
	250 mL chloroform (optima) 697 mL hexane (optima) 1 mL triethylamine (TEA) 1 mL formic acid Solvent B: Methanol
Injector volume	20 µl
Detector Conditions	Temperature: 85°C Airflow rate: 1.8 L/min Time Constant: 1 second Impactor: Off
Time Program	The compound of interest is eluted under isocratic conditions at a flow rate of 1.40 mL/min with 100% mobile phase A for 10 minute. To clean the column, the flow rate is increased to 2.00 mL/min with 100% mobile phase B for 3 minutes. From 13 to 19 minutes the mobile phase is switched to 100% Solvent A to equilibrate the column while maintaining the flow rate at 2.0 mL/min. At 19.01 minutes, the flow rate is reduced to the initial flow rate of 1.40 mL/min

A standard curve was generated from a series of four to five standards which contained a known amount of carboquat (certified Manufacturing Use Product supplied by the Sponsor), a known volume of chloroform:methanol (5:1), and the internal standard (DoDAB) at a concentration of 200 ppm. The Manufacturing Use Product was used because a pure analytical standard for carboquat was not available. Serial dilutions were performed such that the internal standard concentration was maintained at 200 ppm. A standard curve was generated by plotting the area of carboquat/ DoDAB obtained from the chromatograms versus the corresponding carboquat concentration. The r^2 values were greater than 0.995 for all curves generated.

Carboquat concentrations in the sample extracts were determined using the regression equation obtained from a series of standards. The amount of carboquat recovered was then calculated by multiplying its concentration (µg/mL) by the final volume of the extract (mL) and appropriate dilution factor (if any).

There are no graphical standard curves provided in this report. However, the standard curve data is provided in the raw data and is acceptable.

II. RESULTS AND CALCULATIONS

The method validation recoveries were calculated using the following equation as presented in the study report:

$$\text{Percent Recovery (\%)} = \frac{\text{Amount of carboquat recovered (µg)}}{\text{Fortification amount (µg)}} * 100 \qquad \textbf{Eq. 1}$$

The recoveries calculated are presented in Table 12.

Table 12: Percent Recovery for Fortified Matricies

Matrix	Amount Fortified (ug)	% Recovery	Average % Recovery
Human Palm	58.2	78.4	78.0±5.9
	231.2	80.7	
	566.6	74.8	
Dry Wipe	24.9	94.7	93.3±4.8
	256.1	90.8	

	639.8	94.5	
Wet Wipe	25.9	93.5	94.1±3.9
	253.4	93.5	
	622.0	93.5	

The method detection limit (MDL) and the limit of quantitation (LOQ) were calculated by the Study Author using the following equations:

$MDL = t_{(n-1, 1-\%)} \times SD$ **Eq. 2**

Where:
$t_{(n-1, 1-\%)} = 3.143$ for n =7 and % = 0.99
SD = standard deviation of 7 replicate analysis of samples fortified at concentrations near the estimated LOQ.

$LOQ = 2 \times MDL$ **Eq. 3**

Table 13 reports the raw data values that were collected to validate the analytical methodology. In addition, the standard deviation values reported in Table 14 for the low fortification levels were used in Equation 2 to calculate the MDL. This then allowed for the calculation of the LOQ. The method detection limit (MDL) values and limits of quantitation (LOQ) values are reported in Table 3 for all of the matrices, and the values in µg/cm^2 are based on a theoretical surface area of 500 cm^2.

Table 13. Method Validation Recoveries

Matrix	Fortification Level	Recovered Amount (µg)	Recovery (%)	Average Recovery By Fortification Level (%)	Standard Deviation (%)	Overall Average Recovery (%)	Standard Deviation (%)
Palm	Low - 58.2 µg	44.6	76.6	78.4	6.3	78.0	5.9
		42.6	73.2				
		48	82.5				
		47.9	82.3				
		40.1	68.9				
		51.1	87.8				
		45	77.3				
	Mid - 231.2 µg	176.6	76.4	80.7	5.3		
		180.8	78.2				
		179.4	77.6				
		189.8	82.1				
		186.8	80.8				
		180	77.9				
		212.5	91.9				
	High -566.6 µg	409.8	72.3	74.8	5.3		
		444.8	78.5				
		451.6	79.7				
		425.1	75.0				
		460.5	81.3				
		378.7	66.8				
		397.5	70.2				
Dry Wipe	Low - 24.9 µg	22.7	91.2	94.7*	5.4	93.3	4.8

		23.1	92.8				
		25.5	102.4				
		21.7	87.1				
		23.3	93.6				
		25.2	101.2				
		23.6	94.8				
	Mid - 256.1 µg	232.4	90.7	90.8	4.6		
		237.8	92.9				
		231.4	90.4				
		212.5	83.0				
		250.8	97.9				
		226	88.2				
		236	92.2				
	High - 639.8 µg	584.7	91.4	94.5	3.8		
		624.5	97.6				
		598.9	93.6				
		579.8	90.6				
		604.2	94.4				
		592.1	92.5				
		648.1	101.3				
Wet Wipe	Low - 25.9 µg	25.9	100.0	93.5*	3.9	94.1	3.9
		24.6	95.0				
		23.7	91.5				
		24.2	93.4				
		24.7	95.4				
		22.6	87.3				
		23.8	91.9				
	Mid - 253.4 µg	244	96.3	93.5	3.6		
		246	97.1				
		229.5	90.6				
		224	88.4				
		233.7	92.2				
		234.1	92.4				
		247.6	97.7				
	High - 622 µg	575.7	92.6	95.4	4.3		
		618.9	99.5				
		571.9	91.9				
		597.8	96.1				
		552.3	88.8				
		619.2	99.5				
		616.6	99.1				

The numbers in this table were calculated by the Agency. The numbers indicated with an * were different from the number provided in the study report. The study author acknowledged that rounding would most likely show some difference in numbers, and these are in the tenths place, so the data provided in the study report is assumed accurate by the Agency.

Table 14: MDL AND LOQ DETERMINATION

				ug/sample		ug/cm^2 [a]	
Matrix	**Fortification Level**	**Average**	**StDev**	**MDL**	**LOQ**	**MDL**	**LOQ**
Palm	Low (58.2 ug)	45.6	3.69	11.6	23.2	0.023	0.046
Dry Wipe	Low (24.9 ug)	23.6	1.35	4.2	8.5	0.008	0.017
Wet	Low (25.9 ug)	24.2	1.02	3.2	6.4	0.006	0.013

Wipe							

a: To convert from ug/sample to ug/cm^2 the sample area was assumed to be 500 cm^2

III. CONCLUSIONS:

The results presented for this MRID support that the analytical methodology selected for quantifying carboquat is sufficient.